VOLUME XXII JULY, 1939 NUMBER 7

JOURNAL OF DAIRY SCIENCE

Contents

A comparison of alfalfa silages prepared by the A.I.V. and molasses methods. D. M. HEGSTED, F. W. QUACKENBUSH, W. H. PETERSON, G. BOHSTEDT, I. W. RUPEL and W. A. KING 489
The determination of blood plasma carotene in the bovine using a photoelectric colorimeter. L. A. MOORE 501
Effect of pasture upon the carotene content of blood plasma of the bovine. L. A. MOORE 513
The use of steapsin in the manufacture of blue cheese. S. T. COULTER and W. B. COMBS 521
Preliminary observations on the treatment of parchment paper with sodium or calcium propionate. H. MACY and J. C. OLSON 527
Comparisons of the inactivation of phosphatase enzyme and the killing of Escherichia-Aerobacter *organisms as criteria of satisfactory pasteurization of ice cream mix.* F. E. NELSON, W. J. CAULFIELD and W. H. MARTIN 535
Study of physical and chemical properties of the adsorption membrane around the fat globules in cream. I. The effect of the adsorption membrane of synthetic creams on curd tension of cow's milk. N. P. TARASSUK and L. S. PALMER 543
Inadequacy of a whole milk ration for dairy calves as manifested in changes of blood composition in other physiological disorders. G. H. WISE, W. E. PETERSEN and T. W. GULLICKSON 559
Value of various vitamins and inorganic salts for overcoming symptoms manifested in calves restricted to a whole milk ration. G. H. WISE, T. W. GULLICKSON and W. E. PETERSEN 573
Influences of three methods of cleaning the cream separator and cream storage temperature on the keeping quality of cream. W. J. CAULFIELD and W. H. MARTIN 583
Abstracts of literature A95

Published by the
AMERICAN DAIRY SCIENCE ASSOCIATION

Made in United States of America

JOURNAL OF DAIRY SCIENCE

VOLUME XXII JULY, 1939 NUMBER 7

A COMPARISON OF ALFALFA SILAGES PREPARED BY THE A.I.V. AND MOLASSES METHODS[1]

D. M. HEGSTED, F. W. QUACKENBUSH AND W. H. PETERSON
Department of Biochemistry, College of Agriculture, Madison, Wis.

G. BOHSTEDT, I. W. RUPEL AND W. A. KING
Department of Dairy Husbandry, College of Agriculture, Madison, Wis.

INTRODUCTION

The large losses of nutrients which accompany ordinary haymaking, especially under unfavorable climatic conditions, have led to a search for a more efficient means of preserving forage. Of the various methods which have been tried, ensiling has received the most attention. At the present time several procedures appear to be practical for the ensiling of legumes and grasses. Two of these are the A.I.V. process, developed by A. I. Virtanen (1–2), which involves the addition of a mineral acid solution to the forage as it is ensiled; and the molasses method, apparently first used by Reed and Fitch in 1917 (3), and recently revived by numerous investigators (4–7).

Watson and associates (8–11) have made an extensive study of various methods for ensiling grassland products. Upon the basis of chemical analyses and feeding trials, they concluded that, while slightly lower losses of nitrogen are obtained with the A.I.V. method, the use of molasses is most applicable to English farm conditions. Davies (12) and other European workers (13) reached a similar conclusion from their experiments. Virtanen (14), however, reported the A.I.V. method as the more satisfactory. His conclusion was based upon the analyses of silages produced in small containers in the laboratory. Reports of success with both types of silage have also come from several investigators in this country (4–7, 15–17). The

Received for publication August 8, 1938. The delay in the publication of this paper was occasioned by an error in filing it in our office. We extend our apologies for this regrettable occurrence to the readers as well as the authors.—*Editor.*

[1] Cooperative experiment by the Departments of Biochemistry, Dairy Husbandry, and Agricultural Engineering. Various phases of the work have been done with the advice or supervision of Professors F. W. Duffee, C. A. Elvehjem, E. B. Hart, B. H. Roche and H. Steenbock. Credit is due Mr. G. M. Werner for direct supervision of the feeding of the cows and collection of many of the milk samples.

Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

earlier results obtained at this station with the A.I.V. method were reported in previous publications (16–17). Similar data comparing the A.I.V. and molasses methods are now available. The comparisons were made with respect to the preservation of forage nutrients, and to the quantity and quality of milk produced on rations containing the silages.

PREPARATIONS OF SILAGES

Each year the silages were prepared from first-cutting alfalfa in the early bloom stage. About 30 tons were ensiled by each method in 1935; 20 tons in 1936, and 23 tons in 1937. The method of preparation of the A.I.V. silage was the same as has been described previously (16) except that the alfalfa was chopped and the acid solution was pumped into the silo with a small centrifugal pump, and sprayed on the forage as it came from the blower pipe.

Commercial blackstrap molasses was used in the preparation of the molasses silage. In 1935 the molasses was diluted with an equal volume of water and applied in the same manner as the A.I.V. acid solution. In 1936 the lower half of the silo was filled in the same way, the upper half by applying the undiluted molasses to the alfalfa back of the feed roll of the ensilage cutter. The 1937 silage was also made with undiluted molasses. The application of undiluted molasses is simpler and requires considerably less labor.

The cuttings of alfalfa in 1935 and 1936 were at approximately the same stage of maturity although there was considerable difference in dry matter content. In 1937 the alfalfa was somewhat younger and low in dry matter. These differences provided a comparison of the two methods for ensiling forage of high and low moisture content; and also partially account for the large amount of A.I.V. acid solution required in 1936, 100 liters per ton, whereas 65 liters per ton were sufficient in 1935 and 80 liters in 1937. Although 100 pounds of molasses were used for each ton of alfalfa in 1935, barrel experiments which were run simultaneously (and also the results of other investigators) indicated that less was sufficient; accordingly, the rate was reduced to 65 pounds per ton in 1936 and 1937.

The large amount of drainage from both silos in 1935 and 1937 could not be measured accurately, since a considerable quantity of liquid seeped through the joints around the doors and was lost. Previous experiments had shown the losses of dry matter and nitrogen by drainage to be practically negligible (10). No drainage occurred from either silo in 1936.

ANALYSIS OF SILAGES

Five samples were taken from each silo during the feeding periods. The first was obtained after approximately three feet of silage had been removed, including about 6 to 12 inches of partially spoiled material on the

surface. Subsequent samples were taken at about four foot intervals. Dry matter, pH, carotene, and ammonia nitrogen were determined immediately on all samples and two of the samples from each silo were analyzed for total nitrogen. In addition, fermentation products were determined upon the last samples obtained from the silos in 1935 and 1936. A summary of these results is presented in Tables 1 and 2.

TABLE 1

Chemical analysis of forage

Forage	Dry matter	pH	Total N (dry basis)	NH_3–N	Carotene (dry basis)
	per cent		*per cent*	*Total N per cent*	*Micrograms per gram*
1935					
Green Alfalfa	22.6		2.84	1.9	137
A.I.V. Silage	23.4	3.5	2.75	3.5	216
Molasses Silage	22.3	3.7	2.63	6.2	150
1936					
Green Alfalfa	30.9				195
A.I.V. Silage	25.4	3.8	2.35	5.4	164
Molasses Silage	28.2	4.3	2.13	9.5	91
1937					
Green Alfalfa	20.2				274
A.I.V. Silage	25.1	3.4	3.07	4.2	256
Molasses Silage	26.8	3.9	2.92	8.2	170

TABLE 2

Fermentation products of silage (per cent of dry matter)

Analysis	1935		1936	
	A.I.V. silage	Molasses silage	A.I.V. silage	Molasses silage
Volatile acids, as acetic	1.32	3.81	2.28	2.66
Acetic acid	1.32	3.81	1.89	2.44
Butyric acid	0.0	0.0	0.41	0.22
Non-volatile acids, as lactic	2.14	9.58	7.25	12.30
Lactic acid	0.71	8.15	3.98	8.12
Ethyl alcohol	0.21	0.77	0.32	0.69

Uniformly low values were found for pH and ammonia for all the A.I.V. silages and for the 1935 and 1937 molasses silages. In 1936, however, the upper half of the molasses silage was of only fair quality. The first sample had a pH of 4.6 and contained 21 per cent of the total nitrogen as ammonia. These values were lower in subsequent samples and the bottom half was of high quality with a pH of 4.1 and only 4.5 per cent of the nitrogen as ammonia. In spite of the variation in chemical composition, all of the silages seemed to be of good quality with respect to appearance and odor.

In 1935 and 1936, the carotene determinations were made according to a method similar to that of Peterson, Hughes and Freeman (17a). Both silages showed higher values in 1935 than the alfalfa which was used in their preparation, the values being 137 micrograms per gram for the green alfalfa, 216 for the A.I.V. silage and 150 for the molasses silage. In 1936, however, some carotene was lost from both silages, but especially from the molasses silage. Samples taken near the top of the molasses silo analyzed only 50 micrograms of carotene while those taken from the bottom half, where both pH and ammonia were lower, showed 150 micrograms per gram.

The observations of Quackenbush, Steenbock and Peterson (18) threw considerable doubt upon the accuracy of these figures. These investigators have shown that in carotene analysis the benzine or petroleum ether fractions of the pigments obtained from silages contain considerable amounts of carotenoids other than carotene; that this is especially true of silages prepared with mineral acids. Consequently in 1937, the carotene determinations were made by a modified procedure and carefully checked by chromatographic analysis. Again the A.I.V. silage showed consistently higher values than the corresponding molasses silage, although some carotene, approximately 13 per cent, had been lost in the A.I.V. silage. Forty-three per cent of the original carotene had been lost in the molasses silage.

Total nitrogen determinations showed slightly lower values for the molasses silages than for the corresponding A.I.V. silages. However, these figures must be considered as only approximations since there was considerable variation in individual samples and only two analyses were made upon each silage.

Fermentation products, especially non-volatile acids, were formed in larger amounts in the molasses silages than in the corresponding A.I.V. silages. However, the A.I.V. silage in 1936 contained larger amounts of total non-volatile acids and lactic acid than that prepared in 1935. Formation of butyric acid was completely suppressed in both silages in 1935. Although butyric acid was present in slight amounts in both silages in 1936, there was no objectionable odor.

In 1935 the temperatures were taken by means of thermocouples six feet below the surface and in 1936 and 1937 by thermometers suspended in pipes which were driven down 10 feet into the silage. The bulb of each thermometer was enclosed in a tube of water so that the reading did not change while the thermometer was being withdrawn and read. The curves obtained are shown in Figure 1. In all of the silages there was a rather abrupt rise during the first few days after ensiling although there was no excessive heating in any of the silages. The maximum temperature obtained is probably dependent to a large extent upon the dry matter content of the forage. In 1935 the silos were filled immediately after a heavy rain and very little heating occurred in either of the silages. The other two

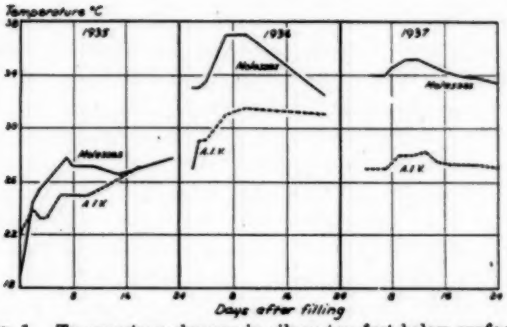

FIG. 1. Temperature changes in silage ten feet below surface.

years the temperatures of the A.I.V. silages were considerably below that of the molasses silages probably because respiration is stopped almost immediately after the acid is applied.

MILK PRODUCTION EXPERIMENTS

Cows from the University herd which had been receiving winter rations for about three months were divided into three lots approximately equal in

TABLE 3

Average daily feed consumption per cow (pounds)

Lot	Alfalfa hay	Corn silage	A.I.V. alfalfa silage	Molasses alfalfa silage	Grain mixture*
1935					
Control	10.7	32.0			10.0
A.I.V.	5.5	20.2	34.4		10.0
Molasses	5.4	18.9		36.4	9.9
1936					
Control	11.3	33.9			11.2
A.I.V.	11.3		30.9		11.0
Molasses	11.7			30.1	11.1
1937					
Control	7.6	45.7			10.0
A.I.V.	7.2		49.5		10.0
Molasses	7.2			49.9	10.0

* Grain mixture:

	Control lot %	*Alfalfa silage lots* %
Ground corn	61.9	69.6
Ground oats	24.7	27.5
Linseed meal	12.4	
Bone meal		1.9
Iodized salt	1.0	1.0

In 1937, the grain mixture for the control lot contained 54.46 per cent ground corn, and 19.8 per cent linseed meal.

The cows on the A.I.V. ration received no salt but were fed 1 oz. of $CaCO_3$–Na_2CO_3 mixture (10:3) per 15 pounds of silage.

weight, age, breed, stage of lactation, and milk and butterfat production. In 1935, each lot consisted of two Holsteins, two Guernseys, one Jersey and one Brown Swiss; in 1936, two Holsteins, two Guernseys and one Ayrshire, with the exception that a Brown Swiss replaced the Ayrshire in the molasses silage lot; and in 1937, two Holsteins, one Guernsey, one Brown Swiss and one Ayrshire. After the cows had been on experiment about six weeks in 1937 the Ayrshire in the A.I.V. lot had to be removed because of a badly infected udder. Since the production, weight, etc., of the Ayrshires in the three lots were similar they were removed from all three lots.

The composition of the rations is shown in Table 3. The hay used the first two years was western grown alfalfa of good quality and contained 27 and 50 micrograms of carotene per gram of dry matter in 1935 and 1936 respectively. In 1937, the alfalfa hay was quite leafy but rather discolored. It was probably more like that fed on the average farm and contained only 17 micrograms of carotene per gram of dry matter. The corn silages were those fed the University herd. In 1935 and 1936 they appeared to be of good quality, but there was considerable difference in their carotene content—37 micrograms per gram of dry matter in 1935 and 126 micrograms per gram in 1936. During the first two months of the feeding experiment in 1937 the corn silage was quite brown and contained only 22 micrograms of carotene. It was better in the lower part of the silo where the carotene ranged from 50 to 60 micrograms per gram of dry matter. The grain mixtures for the three lots were similar except that linseed meal was included in the mixture for the control lots to bring the protein content of the ration to the level of the legume silage rations. In 1937, when rather large amounts of corn silage and small amounts of alfalfa hay were fed to the control lot, it was necessary to include twenty per cent of linseed meal in the grain mixture.

In 1935, one-third of the corn silage and approximately half of the hay in the control rations were replaced by alfalfa silages in the A.I.V. and molasses lots. In this and the following years replacements were made on the basis of equivalent dry matter. In 1936, alfalfa silage replaced all of the corn silage in these lots. In 1937, the cows were encouraged to eat as much legume silage as possible by gradually decreasing the hay allowance. The average consumption was approximately 50 pounds per day although several animals ate 56 to 58 pounds. In the control ration the hay was also cut down and the corn silage increased so that all of the lots received approximately two-thirds the normal hay allowance.

There was no evident difference in the palatability of A.I.V. or molasses silages but they were not consumed as readily as corn silage. Some difficulty was encountered at first in getting the cows to eat large amounts of the legume silages but this was overcome by gradually substituting for the corn silage until the desired level was reached. As shown in Table 4, the

cows gained in weight each year although no lot showed consistently greater gains than the others.

TABLE 4

Average milk production and changes in live weight (pounds per cow)

Lot	Daily milk production*	Decline in daily milk production*	Initial weight (lbs.)	Gain in weight
1935 (18 wks.)				
Control	27.8	6.2	1061.7	56.3
A.I.V.	29.6	5.0	1029.8	64.8
Molasses	29.2	3.5	1044.8	21.8
1936 (15 wks.)				
Control	33.4	7.9	1088.5	28.6
A.I.V.	29.3	5.3	1064.5	48.8
Molasses	31.2	6.1	1111.1	48.7
1937 (17 wks.)				
Control	28.3	2.1	1112.5	79.2
A.I.V.	28.0	4.0	1113.7	62.7
Molasses	28.2	1.4	1157.7	94.5

* 4 per cent fat corrected milk.

The milk production records are also presented in Table 4. During the three years, the milk and butterfat production was maintained almost equally well by all three lots although there is a slight advantage for the molasses silage lot over the three years.

CAROTENE AND VITAMIN A OF BUTTERFAT

Milk samples from each lot were obtained by pooling the milk from the morning and evening milkings. The carotene and vitamin A of the butterfat from these samples were determined spectroscopically by the method of Semb, Baumann and Steenbock (19). In 1935, only a single analysis at the end of the feeding period was made; in 1936 and 1937 several analyses were made at intervals throughout the period. The data are shown in Table 5. In 1935 and 1936 there was no marked difference in the carotene and vitamin A content of the butterfat from individual groups. The control lot was somewhat lower in both carotene and vitamin A in 1935 and slightly higher in both of these constituents in 1936. It was expected that the control lot in 1936 would produce butterfat of rather high vitamin A potency since the corn silage was of exceptionally good quality and high in carotene. However, at the end of the experiment the figures for the three lots were more nearly alike than the averages given in the table. During the last two months the carotene contents averaged 7.2, 7.5 and 6.1 and the vitamin A 8.5, 7.9 and 8.4 micrograms per gram for the control, A.I.V. and molasses lots respectively.

TABLE 5

*Carotene and vitamin A content of butterfat**

Lot	Number of determinations	Carotene	Vitamin A
		micrograms per gram	*micrograms per gram*
1935**			
Control	1	6.5	9.0
A.I.V.	1	7.6	11.0
Molasses	1	7.4	10.0
1936			
Control	6	7.2	8.7
A.I.V.	5	7.0	7.5
Molasses	5	5.6	7.4
1937			
Control	4	4.4	6.7
A.I.V.	4	7.7	11.1
Molasses	4	9.1	11.0

* Average of values obtained after cows had been on experiment three months.
** One determination made at the end of the feeding period.

In 1937 the differences between the control and the legume silage lots were considerable. The results are presented in detail in Figure 2. The vitamin A potency of the butterfat from the control lot fell rather rapidly until corn silage of better quality was used, about December 1, and then rose gradually until the end of the period. Both of the legume silage lots rose most rapidly during the first few weeks but continued to rise throughout the feeding period. The total vitamin A potency of the butterfat from the molasses lot reached a somewhat higher level than that of the A.I.V. lot although the cows in the A.I.V. lot were ingesting considerably more carotene. When the animals in the legume silage lots were placed upon the regular herd ration the carotene and vitamin A of the butterfat fell slowly. The 1937 results are indicative of what could be accomplished through the

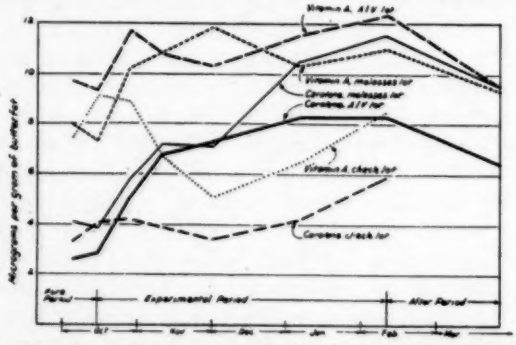

FIG. 2. Effect of ration on the carotene and vitamin A content of butterfat in 1937.

use of legume silages upon the average farm, since the hay and corn silage were quite similar to those ordinarily fed and the carotene and vitamin A contents of the butterfat from the control lot were similar to the average of winter milks which we have analyzed.

GROWTH PROMOTING PROPERTIES OF THE MILK

Composite milk samples were taken daily from each lot. These were mineralized (.5 mg. Fe, .05 mg. Cu, .05 mg. Mn per rat per day) and fed ad libitum to 30 gram male rats in growth experiments as described previously (16–17). The data are given in Table 6. In 1935, gains of 2.8 to 3.0 grams per day were made, which is normal for winter milks. In 1936,

TABLE 6

Growth of rats on mineralized milk (grams gain in 6 weeks)

Lot	1935	1936	1937
Control	128	162	106
A.I.V.	129	148	138
Molasses	120	148	119

the growth was more rapid, approaching that obtained with summer milks. The excellent growth of the rats upon the control milk might be attributable to the high quality of corn silage which was fed. The rats receiving the A.I.V. milk showed somewhat greater gains in 1937. Unfortunately, too few rats were used in that year to warrant a definite conclusion.

Kohler *et al.* (20) have recently used the guinea pig as an experimental animal to determine the growth promoting properties of milks. None of the milks in 1937 were capable of supporting growth of guinea pigs. On all three milks, the animals lost weight rapidly and died in about three weeks.

DISCUSSION

Good preservation of alfalfa was accomplished by both the molasses and the A.I.V. methods of ensiling forage. As measured by the pH, ammonia and carotene data, the A.I.V. method showed some advantages over the molasses method. The carotene figures for the first two years are undoubtedly too high, especially for the A.I.V. silage, but in 1937 accurate determinations showed that the A.I.V. silage contained about 50 per cent more carotene than the molasses silage. The inferior quality of the top half of the molasses silage in 1936 may be attributed in part to the high dry matter content of the original forage. Satisfactory silage was obtained in the bottom of the silo where diluted molasses was used. Certain barrel experiments which we have run also indicate that for best results molasses should be diluted with material fairly high in dry matter.

Virtanen (22) has reported that a considerable portion of the added sugar in molasses silage disappears through alcoholic fermentation and is

thus ineffective in preservation of the material. Our results do not show any more ethyl alcohol in molasses silage than is ordinarily encountered in corn silage (23).

Legume silage rations usually produced butterfat of higher carotene and vitamin A content than hay-corn silage rations. However, when the hay was of superior quality or the corn silage outstandingly high in carotene, the quality of the milk approached that obtained on alfalfa silage rations. Nevertheless, an increase in the carotene content of the ration was not followed by a parallel increase of vitamin A potency in the milk. This lack of response was especially evident in the A.I.V. groups. The A.I.V. silage contained appreciably more carotene than the molasses silage yet the milks produced therefrom were no more potent than those produced on the molasses silage. The data for 1937 show this most clearly, as in that year the carotene contents of the silages were determined by a more accurate method. The animals receiving the A.I.V. ration ingested approximately one-third more carotene than those in the molasses lot. Nevertheless, the total vitamin A potency of the butterfat from the A.I.V. lot was slightly lower than that from the molasses lot. Watson and Ferguson have also reported that A.I.V. silage was less efficient than either molasses silage or artificially dried grass in increasing the vitamin A potency of the butterfat.

The data on consumption, weight maintenance, and milk production are practically alike for the two alfalfa silages. From the point of view of initial cost of preservation there is probably little difference between the two methods. However, it should be pointed out that at least 75 per cent of the feeding value of the molasses is retained in the silage so that a large part of the initial cost is returnable. How much should thus be credited to the molasses method will depend of course on the feeding value assigned to molasses in comparison with equivalent feeds. Other items of cost in favor of the molasses method are the absence of license fees and cost of lime and soda. Taking various factors into consideration, especially the simplicity of operation, the molasses method appears to be preferable to the A.I.V. method. This conclusion is in agreement with that of Watson and associates who, after extensive experiments with the two methods, decided that for English conditions the molasses method is to be preferred.

Regardless of the method of ensiling, milk production should cost somewhat less with alfalfa silage than with corn silage because of the saving in high protein concentrates such as oil meal. This same advantage would of course be possessed also by alfalfa hay.

To the average farmer the greatest appeal of legume and grass silages lies undoubtedly in freedom from weather hazards during the harvesting of the crop. Absence of fire hazard is another advantage in making silage. On the debit side should be noted a somewhat greater labor cost as compared with hay-making and the necessity for more silos unless alfalfa replaces corn in the farm program.

SUMMARY

Alfalfa silages prepared by the A.I.V. and molasses methods were compared during three years. The comparison was based upon chemical analysis of the product and the quantity and quality of milk produced in feeding trials. Chemical studies showed no pronounced differences, although protein and carotene were better preserved by the A.I.V. method.

Good preservation of alfalfa containing 20 to 25 per cent of dry matter was obtained by both methods. Carotene and protein losses were somewhat larger when the forage contained 30 per cent dry matter.

Rations containing either A.I.V. or molasses alfalfa silage, or corn silage and linseed meal, were equally satisfactory for milk production.

The carotene and vitamin A contents of the butterfats produced on the legume silage rations were high but only in 1937 appreciably different from those produced upon the control ration. It is probable that under average farming conditions the vitamin A potency of the butterfat can be greatly increased by the use of legume silages.

The milks from the three lots had approximately the same growth promoting properties when tested on rats. All of the milks in 1936 were superior to most winter milks. None of the milks in 1937 would support growth of guinea pigs.

Molasses alfalfa silage appears to be equal to A.I.V. alfalfa silage, and conditions of farm practice probably make molasses silage more suitable for general use.

REFERENCES

(1) VIRTANEN, A. I. Die A.I.V. Methode zur Konserveirung von frischen Futter. Schweiz. Landw. Monatesheftе 10: 257–269. 1932.

(2) VIRTANEN, A. I. Five years' experience of A.I.V. fodder. Contributions from Laboratory of Valio, No. 2. 1934.

(3) REED, O. E., AND FITCH, J. B. Alfalfa silage. Kans. Agr. Exper. Sta. Bull. 217: 3–19. 1917.

(4) BENDER, C. B., TUCKER, H. H., KRUEGER, W. C., PROU, K. O., AND FOX, A. S. Molasses hay silage. J. DAIRY SC. 19: 137–146. 1936.

(5) HAYDEN, C. C., PERKINS, A. E., KRAUSS, W. E., MONROE, C. F., AND WASHBURN, R. G. Legume silage for dairy cows. Ohio Agr. Exper. Sta. Bimonthly Bull. Vol. 22, No. 184: 21–27. 1937.

(6) ELLENBERGER, H. B. Work at the Vermont Experiment Station. Hoard's Dairyman 82 (20): 563, 577. 1937.

(7) ELTING, E. C. Molasses as a preserving agent in making soybean silage. J. DAIRY SC. 18: 440. 1935.

(8) WATSON, S. J., AND FERGUSON, W. S. The value of artificially dried grass, silage made with added molasses, and A.I.V. fodder in the diet of the dairy cow, and the effect on the quality of milk, with special reference to the value of the non-protein nitrogen. J. Agr. Sc. 26: 337–366. 1936.

(9) ALLEN, L. A., HARRISON, J., WATSON, J. S., AND FERGUSON, W. S. A study of the chemical and bacteriological changes occurring in grass silage. J. Agr. Sc. 27: 271–293. 1937.

(10) WATSON, S. J., AND FERGUSON, W. S. The chemical composition of grass silage. J. Agr. Sc. 27: 1–42. 1937.

(11) WATSON, S. J., AND FERGUSON, W. S. The losses of dry matter and digestible nutrients in low-temperature silage, with and without added molasses or mineral acids. J. Agr. Sc. 27: 67–107. 1937.

(12) DAVIES, W. M., BOTHAM, G. H., AND THOMPSON, W. B. Grass silage. A comparison of the changes involved in the ordinary, molasses and A.I.V. processes. J. Agr. Sc. 27: 151–161. 1937.

(13) FAGAN, T. W., AND ASHTON, W. M. The preservation of green forage. Welsh J. Agr. 13: 129–143. 1937.

(14) VIRTANEN, A. I. The microbiology of ensilage production. Zentr. Bakt. Parasitenk. 95: 472–477. 1937.

(15) WOODWARD, T. E., AND SHEPHERD, J. B. Methods of making silages from grasses and legumes. U. S. D. A. Tech. Bull. 611. 33 pp. 1938.

(16) PETERSON, W. H., BOHSTEDT, G., BIRD, H. R., AND BEESON, W. M. The preparation and nutritive value of A.I.V. silage for dairy cows. J. DAIRY SC. 18: 63–78. 1935.

(17) PETERSON, W. H., BIRD, H. R., AND BEESON, W. M. Chemical changes in the making of A.I.V. alfalfa silage and nutritive qualities of milk produced therefrom. J. DAIRY SC. 20: 611–623. 1937.

(17a) PETERSON, W. J., HUGHES, J. S., AND FREEMAN, H. F. Determination of carotene in forage. Modification of the Guilbert method. Ind. Eng. Chem., Anal. Ed. 9: 71–72. 1937.

(18) QUACKENBUSH, F. W., STEENBOCK, H., AND PETERSON, W. H. The effect of acids upon carotenoids. Proc. Soc. Biol. Chem., J. Biol. Chem. 123: XCVIII. 1938.

(19) SEMB, J., BAUMANN, C. A., AND STEENBOCK, H. Fat-soluble vitamins XLI. The carotene and vitamin A content of colostrum. J. Biol. Chem. 107: 697–703. 1934.

(20) KOHLER, G. O., ELVEHJEM, C. A., AND HART, E. B. The relation of the "grass juice factor" to guinea pig nutrition. J. Nutrit. 15: 445–459. 1938.

(21) ECKLES, C. H. Legumes, sudan grass and cereal crops for silage. Mo. Agr. Exper. Sta. Bull. 162. 25 pp. 1919.

(22) VIRTANEN, A. I., AND KARSTRÖM, H. The decisive importance of pH in silage problem. Compt. rend. du Lab. Carlsberg. Ser. chim. 22: 528–539. 1937.

(23) PETERSON, W. H., HASTINGS, E. G., AND FRED, E. B. A study of the principal changes which take place in the making of silage. Wis. Agr. Exper. Sta. Res. Bull. 61. 30 pp. 1925.

THE DETERMINATION OF BLOOD PLASMA CAROTENE IN THE BOVINE USING A PHOTOELECTRIC COLORIMETER[1]

L. A. MOORE

Michigan Agricultural Experiment Station, East Lansing, Michigan

The determination of the carotene content of blood plasma is of value in vitamin A studies especially when the entire food source of vitamin A is in the form of carotene as is usually the case with herbivorous animals.

Although there are many pigments in the blood stream the methods generally used for the determination of carotene exclude interference of the bile and respiratory pigments. The pigments extracted from blood serum or plasma by the methods which employ ethyl alcohol for the precipitation of the proteins and ethyl or petroleum ether for extraction, fall in the carotinoid class, the most important being the carotenes and xanthophylls.

Palmer (1) reported that in bovine serum, the carotinoids consist almost entirely of carotene with a very small fraction of xanthophyll. The carotinoids of horse serum also consist almost entirely of carotene whereas chicken serum contains mostly xanthophyll. In the case of human serum carotene accounts for most of the carotenoid fraction although some xanthophylls may be present. Gillam and Ridi (2) found only 5 per cent of the total carotinoids of bovine serum to be xanthophyll. By concentration of the carotene fractions from several liters of cow serum and separation by the adsorption method with alumina they found very small amounts of kryptoxanthin.

Since carotinoid pigments other than carotene are present in bovine and equine serum or plasma in only very small amounts, it would seem that the precipitation of the proteins by means of alcohol and the extraction of the carotene with petroleum ether offers a very simple method of analysis. In other types of blood in which significant amounts of other pigments might be present it would obviously be necessary to make further separations.

Various modifications of this procedure have been used by White and Gordon (3), Palmer and Eckles (4), Patchevsky (5), Daniel and Scheff (6), Gillam and Ridi (2), and Clausen and McCoord (7).

In general the methods used are more time consuming than necessary because they involve repeated extractions to remove all of the carotene. This is not necessary if application is made of the Nernst distribution law by the use of a phase separation. Moreover the availability of the photoelectric colorimeter has greatly improved the measurement of color. For

Received for publication January 12, 1939.

[1] Journal article No. 344 (N.S.) Michigan Agricultural Experiment Station. From a portion of a thesis presented to the Graduate School of Michigan State College in partial fulfillment for the Ph.D. degree.

most of the methods the various factors and limitations have not been studied so that this was one of the main objectives of this investigation.

EXPERIMENTAL METHODS

After trying several methods the following procedure was adopted. Twenty-five to 30 ml. of citrated blood was drawn from the jugular vein and centrifuged to obtain the plasma. Ten ml. of plasma was pipetted into 75 ml. test tubes and 15 ml. of 95 per cent alcohol added from a burette. The tubes were corked and shaken to precipitate the proteins. Five ml. of petroleum ether was then pipetted into the tubes and the tubes tightly corked and placed in cold running tap water having a temperature of about 10° to 12° C. After about five minutes the tubes were shaken vigorously for one minute and again placed in the cold water. This shaking procedure was repeated twice, the tubes being returned to the cold water each time to prevent the loss of petroleum ether. Since the tubes were at room temperature when they were corked a small vacuum was produced upon cooling which tended to tighten the cork in the tube so that there was practically no loss of the solvent.

After the third and last shaking each tube was filled with cold tap water to about 1½ inches from the top to facilitate the separation of the petroleum ether layer. The tubes were immediately recorked and placed in a refrigerator at about 10° below the temperature of the tap water. The tubes were left in the refrigerator for one to three hours to permit the petroleum ether layer to separate after which the carotene concentration was determined. If an emulsion was present in the petroleum ether layer, it could be eliminated in a few minutes by the addition of 0.5 ml. of 95 per cent alcohol. The alcohol was permitted to drop through the petroleum ether layer.

In order to establish the relationship between the amount of carotene extracted by the method just outlined and the total amount present in the sample a procedure was used which was identical with the previous one up to the point where water was added to the tubes. At this stage the tubes were centrifuged and the petroleum ether layer pipetted into a 25 ml. volumetric flask. An additional 5 ml. of petroleum ether was added to the tube and the tube was shaken for one minute. After cooling, the tubes were again centrifuged and the petroleum ether removed as before. This procedure was repeated five times and the extract made up to volume with petroleum ether. The fourth extract would usually be colorless so that it was reasonable to assume that five extractions achieved complete removal of the carotene.

THE PHOTOELECTRIC COLORIMETER

The recent development of the photoelectric colorimeter offers a physical means for measuring the density of color eliminating the shortcomings of the ordinary colorimeter which requires matching depth of color with the eye.

The general principles of the single cell photoelectric colorimeter used in this investigation are relatively simple. A beam of light from a small bulb is passed through a glass filter to limit the light to the desired range of the spectrum. The light then passes through an absorption cell containing the colored solution and falls on a photocell producing a deflection in a galvanometer. The amount of light transmitted through the colored solution is proportional to the ratio of the final to the initial galvanometer deflections. The concentration of the colored substance in the sample can then be read from a chart showing the variation of light transmission with concentration.

The instrument used was the Evelyn micro photoelectric colorimeter (8) in which small amounts of a solution may be used. In this particular instrument the light passes through an open type cell so that various depths of a solution may be used. The depths produced by 0.5 ml., 1 ml. and 2 ml. of solution were used in this study. The open type cell works satisfactorily with light passing up through it when liquids of a low surface tension are used. With liquids of high surface tension such as aqueous solutions, a glass plunger may be used to eliminate the surface tension effect.

Two filters were used in this investigation. The first one, 440, was quite dense so that it was necessary to burn the bulb at almost its maximum in order to get sufficient light through the cell. This made it necessary to replace the bulbs quite often. All the data reported in this paper except as indicated were obtained by the use of this filter. In order to overcome this difficulty a second filter, M 440, was obtained which was less dense and permitted burning the bulb at a much lower intensity. Both filters had maximum transmission of light at a wave length of 440 millimicrons.

The instrument was calibrated by preparing solutions of carotene* of varying concentrations in petroleum ether B. P. 40–60° C. and recording the galvanometer readings for the particular concentration and depth used. The carotene used was not a pure Beta carotene but previous experience had shown it to give curves almost identical with those of pure Beta carotene.

The calibration data may then be transferred to one cycle semi-logarithmic paper by plotting the concentration of carotene against the galvanometer readings. Tables may also be set up by first preparing the graph and interpolating for intermediate points. The latter method effects a large saving of time when considerable work is to be done. From the graphs or tables the concentration of carotene per ml. of blood plasma can be calculated.

EFFECT OF USING PETROLEUM ETHERS OF DIFFERENT BOILING POINTS ON THE AMOUNT OF CAROTENE EXTRACTED

One of the first points to establish was the relationship between the method in which some of the carotene was still present in the hypophase

* Purchased from S. M. A. Corporation.

and the total amount present. In this connection it was expected that the boiling point of the solvent used would have some effect upon the concentration of the carotene because the greater solubility of the shorter chained hydrocarbons in alcohol-water solutions would reduce the volume of the epiphase. Consequently four petroleum ethers with boiling points of 20–40° C., 40–60° C., 68–70° C. and 73–76° C. were selected for the comparison.

In the adopted method two ml. of the epiphase was pipetted into 10 ml. volumetric flasks and made up to volume with each solvent. In this way all dilutions were the same so that the galvanometer readings could be compared directly. Filter M 440 was used in collecting these data. The readings were taken with 1 ml. samples and the results are shown in Table 1.

It will be noted that the galvanometer readings for the two lower B. P. petroleum ethers are much lower than those obtained by the total extraction method indicating that per milliliter of extract the carotene content was higher. These readings were sufficiently different to give values 10 to 12 per cent too high. When the galvanometer readings were converted to carotene values the following factors were obtained: for 20–40° C., B. P. .894; 40–60° C., B. P. .913; 68–70° C., B. P. .988; 73–76° C., B. P. 1.001.

The differences of the means were tested for significance by the Fisher method (9). The t values for the differences between the means obtained by the total extraction method and the phase separation with petroleum ethers with boiling points of 20–40° C. and 40–60° C. were 9.8 and 8.0 respectively whereas the t value from Fisher's tables for 10 degrees of freedom is 3.169 at the one per cent point. These indicate highly significant differences. There was no significant difference when the higher B. P. petroleum ethers were used. The difference between the means of the two higher B. P. petroleum ethers was significant. The t value was 2.91 as compared with a value of 2.23 at the 5 per cent point for 10 degrees of freedom.

It was observed that solvents of differing boiling points gave different center settings. For instance, a petroleum ether with a boiling point of 20–30° C. gave a center setting of 48 whereas one with a boiling point of 73–76° C. gave a center setting of 52.4. However, when solutions of carotene of the same concentration were made with the different solvents the same galvanometer readings were obtained. Consequently the calibration data obtained with a solvent of one boiling point may be used with a solvent of a different boiling point.

EFFECT OF TEMPERATURE DURING EXTRACTION

In order to study the effect of temperature on the amount of carotene extracted by the solvent, determinations were made at 3 different temperatures using petroleum ether with boiling point of 40–60° C. One sample was extracted at room temperature, 25° C., one at that of tap water (10° C.) and one at −24° C. In the latter case the proteins were precipitated

TABLE 1

Effect of boiling point of solvent upon carotene extraction

Sample No.	Total extraction 20–40° C.	Adopted method			
		20–40° C.	40–60° C.	68–70° C.	73–76° C.
	Galvanometer readings				
1	67.8	65.5	64.5	68.7	68.5
2	28.5	25.8	26.0	28.9	29.4
3	79.5	77.8	77.8	79.8	80.0
4	71.5	69.0	70.5	71.0	72.1
5	60.0	58.0	59.0	60.5	61.7
6	70.5	68.0	69.1	70.7	71.2
7	63.7	59.0	61.0	63.0	62.8
8	19.5	17.0	17.5	19.1	19.5
9	27.5	24.5	24.8	26.8	27.0
10	63.0	61.2	61.5	63.4	63.8
11	32.5	28.5	29.0	31.2	31.4

at room temperature, petroleum ether added and the tubes kept at −24° C. for about one-half hour. They were then shaken for three one minute periods, after which water was added with a temperature of about 0° to 1° C. The tubes were left in the cold until the petroleum ether layer had separated. The determination of the carotene was made at room temperature.

The results shown in Table 2 indicate a correlated agreement at the various temperatures. Using the statistical treatment previously mentioned the t value for the difference between the means at 25° C. and 10° C. was 4.66, for 10° C. and −24° C., 5.36 and for 25° C. and −24° C., 7.04. According to Fisher's tables the t value for 12 degrees of freedom at the one per cent point should be greater than 3.055 so that the means at the various temperatures are significantly different from each other.

TABLE 2

Effect of temperature of extraction upon galvanometer readings

Sample No.	+25° C.	+10° C.	−24° C.
	Galvanometer readings		
1	30.0	31.2	35.0
2	24.0	23.8	28.0
3	82.5	83.3	85.0
4	18.3	19.0	20.5
5	23.5	24.1	25.5
6	19.5	20.9	22.8
7	75.8	75.8	78.0
8	75.0	75.4	77.0
9	29.0	29.5	30.4
10	34.0	34.8	35.0
11	35.0	36.1	37.3
12	84.0	84.5	85.3
13	77.5	77.6	78.4

THE EFFECT OF THE TEMPERATURE OF THE PETROLEUM ETHER EXTRACT AT THE TIME OF PIPETTING INTO THE ABSORPTION CELL

Some of the solutions used to standardize the instrument (crystalline carotene dissolved in petroleum ether B. P. 40–75° C.) were cooled to below – 10° C. The temperature of the solution was allowed to increase and 1 ml. portions pipetted into the absorption cells at – 10, 0, + 10 and + 20° C. respectively. Measurement of the carotene was made immediately after each transfer. The results showed that the readings at the various temperatures checked quite closely.

EFFECT OF USING VARIOUS AMOUNTS OF ALCOHOL FOR PRECIPITATION OF PROTEINS

In order to study the effect of adding various amounts of alcohol, 5, 10, and 15 ml. of alcohol were added to triplicate samples of plasma. The results showed that for the 10 and 15 ml. amounts of alcohol the galvanometer readings checked fairly well whereas in the case of the addition of 5 ml. the results were less consistent. It was also noted that in the 5 ml. tubes an emulsion was often present in the petroleum ether layer.

EFFECT OF TIME OF SHAKING

Triplicate samples were prepared and the length of time of shaking the tubes was varied using 3, 2 and 1 minute periods. The results indicated fairly good agreement between the different times used although the 2 and 3 minute periods gave the most consistent values.

EFFECT OF ADDING ALCOHOL TO PETROLEUM ETHER LAYERS

Since it was necessary in some of the samples to add 0.5 ml. alcohol in order to clear up an emulsion in the petroleum ether layer it was decided to determine whether the use of larger amounts of alcohol would have any effect on the galvanometer reading.

Triplicate samples were prepared and previous to the transfer of the petroleum ether extract to the absorption cell 0.5 ml. and 3 ml. portions of alcohol were added to each set whether an emulsion was present or not. The addition of alcohol had no measurable effect on the galvanometer readings. It was noted, however, that alcohol added when the higher boiling petroleum ethers were used, caused a cloudiness to develop in the epiphase which cleared up only after standing for several hours.

RELIABILITY OF THE METHOD

In order to determine the dependability of the method frequent analyses were made of the samples over a considerable period. The blood was centrifuged immediately after collection and plasma stored in the refrigerator at a temperature of about 2.0° C. Ten ml. of plasma was removed at intervals

and the carotene content determined. This was done 11 to 12 times on each sample over a period of 23 days with filter 440 and over a period of 15 days with filter M 440. Petroleum ether with a boiling point of 40–75° C. was used. The results are shown in Table 3 in which the means, extremes, standard deviations and coefficients of variation are given for each sample.

With filter M 440 the greatest deviation from the mean in 107 determinations exclusive of those obtained with sample C 118 amounted to 7.7 per cent of the mean. With filter 440 of 83 determinations excluding those of A 1, C 118 and C 245, the greatest deviation amounted to 13 per cent of the mean. With these exceptions the results were subject to little variation from day to day.

TABLE 3

Showing variations with statistical constants relative to the determination of carotene in blood plasma

Animal No.	Det. made	Micrograms carotene per ml.				Coef. of Var.	Mean Gal. reading
		Mean	Extremes		Stand. Dev.		
			Upper	Lower			
440 Filter							
	Extract read directly						
A 1	12	0.052	0.092	0.025	±.027	51.9	96.8
G 33	12	0.57	0.65	0.53	±.033	5.8	64.4
C 118	12	0.09	0.12	0.06	±.016	17.7	93.6
A 22	12	1.84	1.92	1.70	±.069	3.8	32.5
A 14	12	2.07	2.21	1.94	±.083	4.0	23.4
C 245	12	2.78	2.90	2.44	±.192	6.9	16.4
	Extract diluted 1–10						
A 15	12	4.56	4.86	3.97	±.218	4.8	70.1
A 7	12	6.94	7.10	6.45	±.187	2.7	59.1
A 18	12	7.09	7.60	6.25	±.380	5.4	58.4
D 11	11	9.17	9.55	8.75	±.310	3.4	50.2
M 440 Filter							
	Extract read directly						
C 118	12	0.15	0.17	0.14	±.009	5.7	90.0
G 33	12	0.52	0.56	0.49	±.021	4.0	69.6
A 22	12	2.21	2.31	2.12	±.064	2.9	27.3
A 14	12	2.29	2.39	2.17	±.062	2.7	26.4
A 26	12	2.68	2.76	2.62	±.046	1.7	22.1
	Extract diluted 1–10						
C 245	12	2.61	2.69	2.49	±.059	2.3	83.3
D 5	12	4.67	4.87	4.46	±.108	2.3	72.0
A 15	12	4.84	4.91	4.70	±.066	1.4	71.2
A 18	12	4.85	4.93	4.70	±.075	1.6	71.2
D 11	10	6.55	6.97	6.09	±.295	4.5	64.1

DISCUSSION OF RESULTS

The results obtained by using solvents of differing boiling points in relation to the total amount of carotene present are very interesting. The data shown in Table 1 indicate quite conclusively that different results may be obtained with solvents of different boiling points. With the lower boiling point petroleum ethers, lower galvanometer readings or higher carotene values were obtained. The differences between the means were statistically significant. With the higher boiling point petroleum ethers the galvanometer readings were very similar.

It must be recognized at the outset that several factors may affect the distribution and concentration of the carotene between the two phases, the alcohol-plasma-water on the one hand and the solvent on the other. One important factor is the miscibility of the two phases and another the distribution ratio of carotene between the two phases. A third is the effect of temperature on each of the others.

The results may be explained by the operation of these factors. In the case of a petroleum ether having a relatively high solubility in the hypophase the volume of the epiphase would be correspondingly low and the concentration of carotene per unit volume relatively higher. The greater solubility of the low boiling point ethers in the hypophase is due to the shorter chains of smaller hydrocarbon molecules. Seidell (10) gives data showing that pentane is considerably more soluble in an alcohol water solution than is hexane, heptane, or gasoline. The petroleum ether with a boiling point of 20–40° C. is probably mostly pentane. Data were not found showing the solubility of these various solvents in a 20–30 per cent alcohol solution which is approximately the concentration after the addition of water in the method used. However, such data would probably be of little value in a system where blood plasma was introduced since the fatty constituents of the plasma would undoubtedly change the solubilities from that of a pure system.

That the amount of carotene extracted with petroleum ether was less than the total amount was shown by the fact that if the hypophase was separated from the epiphase and the epiphase made up to the same volume as in the total extraction method, the galvanometer reading would be larger indicating a lower concentration of carotene. One factor which probably caused the retention of some of the carotene in the hypophase was the presence of some petroleum ether in this phase.

In the case of the petroleum ether with a boiling point of 73–76° C. the galvanometer readings were about the same as for the total extraction.

The results from Table 1 indicate that if petroleum ether is used in a phase separation of the carotene from an alcohol-plasma-water system a factor must be obtained for each petroleum ether with a different boiling point. The results further indicate that if petroleum ether B. P. 73–76° C. is used

no factor is necessary since the results are essentially the same as those obtained by the complete extraction method. However, when emulsions develop with the higher boiling petroleum ethers so that it is necessary to add alcohol, the added alcohol causes a clouding of the epiphase making it necessary to let the sample stand for sometime before reading. When the readings are desired immediately, it would be advantageous to use a lower boiling petroleum ether and employ a factor.

When the effect of the temperature of extraction was studied, the results in Table 2 show that the concentration of carotene in the epiphase was greater at + 25° C. than at + 10° C. or – 24° C. Their means were shown to be statistically different from each other. These results are probably explainable on the basis of the solubility of the petroleum ether in the hypophase at the different temperatures used. According to Seidell (10) 80 per cent alcohol will take up about 10 per cent by weight of pentane at – 30° C. and 21 per cent at + 15° C. No data were given by Seidel at the alcohol concentration used in these studies but as stated before the blood plasma constituents would probably alter the miscibilities from that of a pure system.

The temperature of the extract at the time it was pipetted into the absorption cell had no measurable effect on the galvanometer readings. It is more than likely that due to the small amount of the extract used for the determination and the considerable contact with the pipette and absorption cell fairly uniform temperatures are attained at the time the readings are made regardless of the initial temperature of the extract.

The use of 10 cc. of alcohol for precipitation of the proteins gave as good results as 15 cc. When 5 cc. was used the results were not so consistent. It was found, also, that when 15 cc. of alcohol was used there was less tendency for emulsions to form.

The study of the influence of the amount of shaking indicated that greater consistency in results were obtained with two to three minutes. Although the time of shaking is a relative matter between different individuals proper mixing of the solvent and alcohol-plasma-phase is absolutely necessary in order to get consistent results. Shaking should not be so violent as to cause the formation of an emulsion. The presence of an emulsion in the lower portion of the petroleum ether phase had no effect on the readings. The use of alcohol to clear up an emulsion which might be present also had no effect on the results.

The results in Table 3 show that the method used is consistent from day to day when proper precautions are taken. The data show that there are but small variations from the mean and indicate that the method is quite reliable. The greatest accuracy was apparently obtained with filter M 440 with which the greatest coefficient of variation was 5.7. In this case, however, the mean galvanometer reading was 90.0. Although it is usually

desirable to keep the galvanometer readings between 20 and 90, greater accuracy is probably attainable within a still smaller range such as 30 to 80.

In the case of filter 440 the two greatest coefficients of variation were 51.9 and 17.7. In both instances the galvanometer readings were above 90 which probably accounts for the large variations. The next largest coefficient of variation of 6.9 was obtained when the galvanometer readings were below 20.

An examination of the daily readings from the detailed data from which Table 3 was made further shows that plasma stored at 2° C. did not decrease in its carotene content. As a matter of fact, plasma samples stored as long as six weeks have maintained their carotene content. Studies on whole blood indicated that as long as hemolysis did not occur there was no decrease in carotene.

These studies show that carotene can easily be determined by the phase separation from an alcohol-plasma-water system by means of petroleum ether. The method is simple and many determinations can be made in one day. The results further show that two factors must be taken into consideration, one the boiling point of the solvent and the other the temperature of extraction. These two factors should be standardized. After standardization a comparison should be made with results obtained by a complete extraction of carotene and if necessary a factor calculated. The use of petroleum ether with a boiling point of 73–76° C. and extraction at a temperature of 10–12° C. obviates the use of a factor.

With some determinations a K value is obtained which may be used for calculation purposes when the calibration data forms a straight line plotted on semi-logarithmic paper. However, since the data for carotene do not quite give a straight line it was felt that the use of the plotted curve directly was more accurate, and more rapid.

The author wishes to express his appreciation to Dr. C. A. Hoppert of the Chemistry Department and Dr. W. D. Baten of the Mathematics Department for their assistance in preparing this paper.

SUMMARY AND CONCLUSION

1. A simple phase separation of carotene from bovine plasma has been outlined.

2. When petroleum ether is used as the solvent the boiling point and temperature of extraction are important factors in the amount of carotene extracted.

3. When petroleum ether with a boiling point of 73–76° C. was used and the extraction carried out at 10–12° C. the epiphase gave true carotene values.

4. When the phase separation method is used, the relationship of the

amount of carotene in the epiphase to the total amount present in the sample should be established.

5. The temperature at time of pipetting into the absorption cell under ordinary laboratory conditions had no measurable effect on the galvanometer readings.

6. The use of 15 ml. of a 95 per cent ethyl alcohol for precipitation of the proteins from 10 ml. of plasma was found to be advantageous.

7. Shaking the tubes for two to three minute periods gave more consistent results than shaking for one minute.

8. The addition of 0.5 ml. of alcohol to destroy emulsions had no effect on the determination.

9. The method was shown to give consistent results from day to day.

10. Plasma stored at 2.0° C. showed no loss of carotene over a period of three weeks.

11. The use of the phase separation method in conjunction with the photoelectric colorimeter offers a simple and rapid procedure for the determination of carotene in bovine blood plasma.

REFERENCES

(1) PALMER, L. S. Carotinoids and related pigments. Pp. 128–130. Chemical Catalog Company, New York. 1922.

(2) GILLAM, A. E., AND RIDI, M. S. E. CCXCIII. Carotinoids and vitamin A in cows' blood serum. Biochem. J. 29: 2465–2468. 1935.

(3) WHITE, F. D., AND GORDON, E. M. Estimation of serum carotene. J. Lab. Clin. Med. 17: 53. 1931.

(4) PALMER, L. S., AND ECKLES, C. H. Carotin—The principal natural yellow pigment of milk fat: its relations to plant carotin and the carotin of the body fat, corpus luteum and blood serum. III. The yellow lipochrome of blood serum. J. Biol. Chem. 17: 223. 1914.

(5) PATCHEVSKY, P. The determination of the carotinoid contents of blood tissues and fluids in daily clinical practice. Bull. Soc. Chem. Biol. 17: 1187–93. 1935.

(6) DANIEL, E., AND SCHEFF, G. J. An Improved method for determination of blood carotene. Proc. Soc. Exp. Biol. & Med. 33: 26–30. 1935.

(7) CLAUSEN, S. M., AND MCCOORD, A. B. The determination of carotene and xanthophyll by a single distribution between liquid phases. J. Biol. Chem. 113: 89–104. 1936.

(8) EVELYN, K. E., AND CIPRIANI, A. J. A photoelectric microcolorimeter. J. Biol. Chem. 117: 365–369. 1937.

(9) FISHER, R. A. Statistical methods for research workers. 6th ed. Pp. 126–127. Oliver and Boyd.

(10) SEIDELL, A. Solubilities of inorganic and organic compounds. 2: 1200–1202. D. Van Nostrand Co., Inc. New York. 1928.

EFFECT OF PASTURE UPON THE CAROTENE CONTENT OF BLOOD PLASMA OF THE BOVINE

L. A. MOORE

Michigan Agricultural Experiment Station, East Lansing, Michigan

Palmer and Eckles (1) demonstrated in 1914 that there was a relationship between the carotene content of the feed consumed and the carotene content of the blood serum. Gillam and Ridi (2) reported the average carotene content of blood sera of cows on winter feed to be 0.4 mg. per 100 ml. as compared to a value of 1.11 on summer feed. Semb, Baumann and Steenbock (3) found that the feeding of A.I.V. silage to 12 cows on winter feed produced an increase from an average value of 5.2 micrograms of carotene per ml. of plasma to 8.8 micrograms in 6 weeks. Since the data in the literature are rather limited as to the effect of pasture on the carotene content of the blood plasma of the bovine, it was decided to study this effect.

EXPERIMENTAL

In the spring of 1937 and 1938, just previous to pasture season, cows producing varying amounts of milk, and heifers were chosen from the Brown Swiss, Holstein, Ayrshire, Jersey and Guernsey breeds of the college dairy herd. In 1938 a few dry cows were included in the breed groups. Blood samples were drawn from the jugular vein at various periods previous to and after turning to pasture. Plasma carotene determinations were made according to a method (4) previously outlined. Extraction was made with petroleum ether B. P. 40–60° C. For this solvent the factor .913 was used as explained in the previous paper. The results in 1937 were obtained by the use of the Lovibond Tintometer whereas those in 1938 were obtained by the use of a photoelectric colorimeter.

In 1937 the cows were turned to pasture on May 10th and the heifers on May 17th. In 1938 the cows and heifers were turned out on various dates from April 19th to May 14th, and the nature of the pasture varied to some extent. However, at this season of the year all plants contain large amounts of carotene so that the types of the pastures themselves should have played little part in affecting the level of blood carotene. In 1938 the cows were fed some silage after turning to pasture because of the necessity of emptying a silo.

Blood samples were also drawn from a Holstein cow which had been on a low carotene ration but had received vitamin A by the daily administration

Received for publication January 12, 1939.

Journal article No. 345 (N.S.) Michigan Agricultural Experiment Station. From a portion of a thesis presented to the Graduate School of Michigan State College in partial fulfillment for the Ph.D. degree.

TABLE 1

Carotene content of blood plasma of cows before and after turning to pasture in 1937

Animal No.	Age yrs.	Daily milk lbs.	Micrograms per ml.				
			Before pasture		After pasture		
			10 days	5 days	2 days	9 days	16 days
Brown Swiss							
239	5	59	2.6	2.6	4.6	9.1	17.3
238	5	43	3.2	3.2	4.1	8.2	15.5
230	3	32	3.4	4.6	4.3	10.0	16.4
240	5	27	2.6	2.7	3.4	10.0	13.2
Holstein							
197	5	72	3.2	3.7	4.6	10.0	17.1
253	3½	54	3.0	4.1	6.4	8.2	19.6
254	3	38	3.2	2.8	4.6	11.0	17.0
186	7	36	3.8	3.0	3.7	8.2	11.9
207	5½	8	2.1	2.5		2.5	6.7
Ayrshire							
134	6	67	2.8	2.3	6.4	10.0	22.8
142	3½	46	5.4	4.1	8.2	11.0	21.0
150	2½	43	2.1	2.2	4.3	10.0	20.1
Jersey							
116	3	29	4.8	4.1	7.3	19.2	23.7
113	3	26	5.1	5.8	8.2	20.1	20.1
115	3	23	4.8	3.7	7.3	15.5	21.0
107	4	22	4.1	3.2	7.3	12.8	22.8
95	5½	17	3.3	3.7	7.3	13.7	15.5
Guernsey							
4	11½	42	6.8	5.0	10.0	19.2	28.8
42	3½	38	8.7	7.5	11.0	24.6	28.8
36	3	27	6.4	6.4	10.0	21.9	25.6
48	2½	27	6.8	4.3	6.4	14.6	27.4

of 40 ml. of cod liver oil. This cow was three years old and had been raised on skim milk, corn starch, bran and cod liver oil with wood shavings as a roughage. She was given 40 pounds of fresh cut alfalfa in the morning at the time this experiment was carried out and had consumed about one-half of it during the first half hour. Each day thereafter she consumed about 80 pounds per day. This freshly cut alfalfa contained about 190 micrograms of carotene per gram on the dry basis so that the intake of carotene was about 2 grams per day. Blood samples were drawn every hour for 19 hours and then intermittently up to 168 hours after the feeding of freshly cut alfalfa

hay. In this way the first effect of the consumed carotene on the level of plasma carotene was obtained.

RESULTS

The results obtained are shown in Tables 1 to 4. In Table 3, which gives the 1938 results, the last sample was drawn at periods varying from one day to a week before the animals were placed on pasture and those taken previously were drawn at weekly intervals.

The animals of each breed have been arranged according to decreasing milk production in Tables 1 and 3. The figure for milk production was the approximate amount after one week on pasture. The breeds have also been arranged approximately according to the increasing carotene content of the plasma.

An examination of the results in Tables 1, 2 and 3 shows that there are two factors which control directly or indirectly the level of plasma carotene, one, the breed, and the other, the amount of milk being produced or the stage of lactation.

The results show that the breeds rank in total amount of plasma carotene approximately in the order in which they are listed in the tables, that is, the Brown Swiss plasma contained less carotene under the same conditions than

TABLE 2

Carotene content of blood plasma of heifers before and after turning to pasture in 1937

Animal No.	Age yrs.	Micrograms per ml.			
		Before pasture		After pasture	
		10 days	3 days	4 days	10 days
Brown Swiss					
233	2	1.3	1.6	4.5	3.2
234	1¾	1.6	0.8	6.4	4.3
Holstein					
265	2	1.1	1.6	4.1	3.7
262	2¼	1.1	2.5	2.7	2.8
Ayrshire					
156	1¼	1.0	0.6	6.7	6.7
154	1¾	2.5	1.4	6.0	6.4
Jersey					
80	1¼	2.5	1.3	8.2	11.0
84	1¼	3.2	1.6	11.9	11.0
Guernsey					
55	1¼	3.3	3.2	8.7	16.4
54	1¾	4.1	2.8	14.6	21.9

TABLE 3

Carotene content of blood plasma of cows and heifers before and after turning to pasture in 1938

Animal No.	Age yrs.	Daily milk lbs.	Micrograms per ml.					
			Before pasture		After pasture			
			7–14	1–7	7 days	14 days	28 days	56 days
Brown Swiss								
239	6	55	2.9	2.7	9.1	8.7	11.8	14.3
238	6	43	3.3	3.3	9.9	11.5	13.8	13.9
240	6	21	3.7	3.7	8.7	10.0	12.8	12.7
230	4	13	3.0	3.0	6.0	6.8	9.6	—
303	1½	—	2.1	2.0	3.3	3.9	4.1	—
305	1½	—	1.4	1.7	1.7	2.6	2.6	—
Holstein								
258	3½	60	2.9	2.8	—	11.1	14.4	20.5
186	8	41	4.2	3.8	—	12.3	15.5	15.8
256	4	22	2.3	2.4	5.2	6.4	7.9	—
220	6	12	2.7	2.9	5.7	6.4	8.8	6.6
278	1½	—	2.3	2.1	5.0	7.1	7.0	—
279	1½	—	2.5	2.6	6.1	7.1	7.4	—
Ayrshire								
156	2	37	4.4	5.0	10.9	12.1	14.4	16.5
157	2	36	3.0	3.1	10.0	12.7	13.7	19.1
141	5	10	—	3.2	7.1	9.6	10.0	—
134	7	—	—	2.3	6.8	8.1	10.2	5.0
161	1½	—	2.5	3.6	5.8	6.0	6.3	—
160	2	—	1.9	1.9	3.5	4.8	5.8	—
Jersey								
117	4	36	4.0	4.8	15.7	16.7	19.7	23.3
111	5	31	3.1	4.4	10.3	10.4	14.2	13.7
116	4	14	—	3.7	11.6	15.1	17.7	13.2
114	4	—	2.5	3.0	8.7	8.7	12.8	4.9
90	1½	—	1.6	3.2	5.2	7.6	13.1	—
84	1½	—	2.5	1.8	5.3	6.0	8.9	—
Guernsey								
41	4½	36	6.6	6.8	—	20.8	25.6	23.6
47	3½	32	7.2	7.5	—	28.6	32.0	21.9
44	4½	17	—	5.8	16.6	19.1	21.1	18.3
25	6	—	—	5.8	9.1	14.7	19.0	24.3
3	1½	—	3.7	4.0	9.5	12.1	17.3	—
6	1½	—	5.5	5.5	12.2	16.1	16.1	—

the Jersey and Guernsey breeds. There appeared to be little difference between the Brown Swiss, Holstein and Ayrshire breeds, although the Ayrshires in a few instances gave higher values.

With respect to milk production within the breed the higher the production the higher the carotene content of the plasma after turning to pasture. The heifers were still somewhat lower and in Table 3 are shown to be lower than the dry cow included in three of the breed groups. However, while the animals were on winter feed this relationship did not hold.

TABLE 4

Carotene content of blood plasma of a cow fed freshly cut alfalfa

Hours after feeding	Micrograms/ml.	Hours after feeding	Micrograms/ml.
0	0.19	14	0.26
½	0.21	15	0.33
2	0.21	16	0.30
3	0.20	17	0.31
4	0.20	18	0.33
5	0.25	19	0.33
6	0.18	22	0.49
7	0.22	28	0.64
9	0.18	36	1.21
10	0.22	48	2.74
11	0.22	120	6.85
12	0.25	168	8.49
13	0.24		

The results in Table 4 show that the first rise in plasma carotene which might be attributed to the high intake of carotene was at 15 hours. This was followed by a very definite rise at 22 hours. The feces at the end of 18 hours were definitely green so that the rise at 15 hours was probably significant. The rise in plasma carotene continued until at the end of the first week (168 hours) the plasma carotene level was about the same as that of Holstein cows after one week on pasture.

DISCUSSION

The results clearly indicate that there are distinct breed differences in the level of plasma carotene under both winter and summer feeding conditions. The breeds ranked according to increasing plasma carotene content in the following order: Brown Swiss, Holstein, Ayrshire, Jersey and Guernsey. However, the differences between the Brown Swiss, Holstein and Ayrshire breeds were small. These results show a relationship with those obtained by other investigators in which the butterfat of the Jersey and Guernsey breeds was found to contain more carotene than that of the Holstein and Ayrshire breeds under the same conditions. In other words, the higher the carotene content of the plasma the higher is the carotene content of the butterfat produced, although the total vitamin A activity of the butterfat of the different breeds remains essentially the same under similar feeding conditions. One might reason, therefore, that the true vitamin A content of blood plasma varies inversely with the carotene content under the same feeding conditions.

Accordingly the true vitamin A content of Holstein plasma would be higher than that of the Guernsey whereas the carotene content would be correspondingly lower.

The reasons for breed differences in these respects are not clear. It may be that the carotene concentration of the blood plasma is governed by differences in absorption or by variations in ability of the liver to convert the carotene to vitamin A. Fasold and Heidemann (5) reported that in goats 8 days after thyroidectomy, the milk fat which is normally white, had a yellow color. The yellow color was shown to be produced by carotene. Murlin (6) has recently reviewed the relationship between vitamin A and thyroxin. Many reports are also present in the literature that diabetics show an increased carotene content of the blood serum (7).

There also appears to be a definite relationship within the breed between milk production and the total increase in plasma carotene after turning to pasture. In other words, the greater the milk production the greater the increase in plasma carotene. The reason for such an increase is not apparent. One might suggest that it was due to greater total intake of carotene associated with the consumption of larger amounts of pasture for the heavier producing cows. This reasoning has some support in the fact that on winter feed the plasma carotene values for the milking cows do not vary with milk production since it is the usual herd practice to feed about the same amount of roughage to all cows.

The plasma carotene values for the heifers of each breed under similar conditions were definitely lower than the values for the milking cows of the same breed.

The results for the two years are on the whole in agreement although those of 1937 were obtained by the use of the Lovibond tintometer and are therefore much less accurate. Higher plasma values for both Ayrshire cows and heifers were secured in 1937 than in 1938. The rise of plasma carotene in 1938 appeared to be a little slower than in 1937. This was probably due to the fact that the cows received some silage in the barn and did not consume as much pasture as in 1937.

It is not certain whether maximum values were obtained in these results although in some instances there was some decrease after 56 days. However, it would be reasonable to assume that maximum values had been reached after this period.

The results obtained with the cow fed the freshly cut alfalfa showed that the first rise occurred approximately 15 hours after the initial consumption of the alfalfa. The rate at which the carotene rises in the blood plasma probably fluctuates with the rate of food passage which varies considerably (8). Using these data in conjunction with those in Tables 1, 2, and 3 one would be led to the conclusion that the greatest rate of rise in carotene concentration of the blood plasma occurred during the first week, after which further increases were at a slower rate.

SUMMARY AND CONCLUSIONS

1. Different breeds kept under similar conditions of feeding ranked in increasing order of plasma carotene values as follows: Brown Swiss, Holstein, Ayrshire, Jersey and Guernsey. There was little difference between the first three.

2. Within the breed there was little difference in the level of plasma carotene values among cows producing milk under winter conditions.

3. Heifers within the breed had definitely lower plasma carotene values than producing cows under both winter and pasture conditions.

4. Cows producing the larger amount of milk within the breed in general showed a greater increase in the level of plasma carotene when they were turned to pasture.

5. One cow with a very low initial plasma carotene value showed an increase within 15 hours after being fed freshly cut alfalfa.

The author wishes to express his appreciation to Dr. C. A. Hoppert of the Chemistry Department for his assistance in preparing this paper.

REFERENCES

(1) PALMER, L. S., AND ECKLES, C. H. Carotene the principal natural yellow pigment of milk fat. Mo. Agr. Exper. Sta. Res. Bull. 12. 1914.

(2) GILLAM, A. E., AND RIDI, M. S. E. CCXCIII. Carotinoids and vitamin A in cow's blood serum. Biochem. J. 19: 2465–2468. 1935.

(3) SEMB, J., BAUMANN, C. A., AND STEENBOCK, H. Fat soluble vitamins. XLI. The carotene and vitamin A content of colostrum. J. Biol. Chem. 107: 697–703. 1934.

(4) MOORE, L. A. The determination of blood plasma carotene in the bovine using the photoelectric colorimeter.

(5) FASOLD, H., AND HEIDEMANN, E. R. Uber die Gelbfärbung der Milch Thyreopriver Ziegen. Zschr. f. d. ges. Exper. Med. 92: 53–56. 1933.

(6) MURLIN, J. R. Vitamins and hormones. J. Am. Diet. Ass. 14: 397–411. 1938.

(7) STEUCK, G. H., FLAUM, G., AND RALLI, E. P. The serum carotene in diabetic patients. J. Am. M. Ass. 109: 343–344. 1937.

(8) MOORE, L. A., AND WINTER, O. B. Rate of passage of inert materials through the digestive tract of the bovine. J. DAIRY SC. 17: 297–305. 1934.

THE USE OF STEAPSIN IN THE MANUFACTURE OF BLUE CHEESE

S. T. COULTER AND W. B. COMBS
Division of Dairy Husbandry, University of Minnesota, St. Paul

Blue cheese made from normal cows' milk, even under the most favorable conditions, must be held approximately six months before enough of the characteristic flavor has developed for the cheese to be marketable. The length of the ripening period is a serious deterrent to extensive commercial manufacture of the cheese in this country.

Since the work of Currie (1) fat hydrolysis by a water-soluble lipase produced by the mold, *Penicillium roqueforti*, has been considered the chief factor in the development of the typical flavor of Roquefort-type cheeses. Lane and Hammer (2) were able to shorten the ripening period by homogenization of the milk from which the cheese was manufactured. They attributed the quicker ripening of homogenized milk cheese to the natural milk lipase, the action of which is accelerated by homogenization. They found it necessary to homogenize the milk at temperatures below 115° F. if the benefits of homogenization in shortening the ripening period were to be realized to the fullest extent. This is compatible in some respects with the work of Sharp and de Tomasi (3) showing that non-lactic acidity and bitterness did not develop in cream if the milk before separation was flash heated to 110° F. (43.3° C.). It was found, however, that if the cream was homogenized the milk had to be heated to 140° F. (60° C.) in order to prevent the lipase action in the cold cream.

Various investigators have shown that the ripening of cheese of the Cheddar type could be hastened by increasing the amount of rennet used. Orla-Jensen (4) found that in Emmental cheese, trypsin caused much more rapid proteolysis than either pepsin or galactase. Wojtkiewicz and Inikkoff (5) attempted to hasten the ripening of cheese by adding pepsin, trypsin, erepsin and rennet separately and in various combinations. Rennet and trypsin were found to hasten the ripening of the cheese. The cheeses to which trypsin was added had a bitter flavor. Better results, from a flavor standpoint, were secured when pepsin was added in combination with trypsin, although some bitterness was evident. Freeman and Dahle (6) experimenting with the ripening of Cheddar cheese found that additional amounts of pure rennin increased the rate of proteolysis. This was also true for pepsin, particularly at the beginning of the ripening period, and an aged product with an appreciably higher flavor score was obtained. Trypsin

Received for publication January 13, 1939.
Journal Series Paper 1683, Minnesota Agricultural Experiment Station.

markedly increased the rate of proteolysis during the early stages of the ripening period, but cheese with a lower flavor score was obtained.

EXPERIMENTAL

Since fat hydrolysis appears to be essential for the development of the characteristic flavor in the Roquefort-type cheese, the addition of enzyme preparations containing lipase appeared to offer some promise of shortening the ripening period.

In March, 1935, a small quantity of milk was manufactured into Blue cheese with commercial steapsin (Pfanstiehl) added to the curd at the rate of two grams per 100 pounds of milk. The resulting cheese developed the characteristic flavor of a Roquefort-type cheese within three months. The control cheese did not have the same intensity of flavor even after six months. Subsequently, more than twenty-five batches of cheese have been made with steapsin added in various amounts, either to the milk before renneting or to the curd before hooping, for all or part of the cheese in each batch. The quantity of the enzyme preparation varied from 0.1 gram to 2 grams per 100 pounds of milk.

The addition of steapsin to the milk or to the cheese curd before hooping at the rate of 1 gram per 100 pounds of milk was found to be sufficient for the development of the characteristic flavor in less than five months. The control cheese did not develop the same intensity of flavor until ripened for more than one year. Smaller amounts of the enzyme preparation were proportionately less effective. The flavor and appearance of the "enzyme" cheese was characteristically different from that of the normal cheese.

Stärkle (7) has expressed the opinion that the formation of methyl ketones by the breakdown of certain fatty acids is responsible for the characteristic aroma of mold-ripened cheese. Hammer and Bryant (8) in a later study have concluded that methyl-n-amyl ketone is an important constituent of Blue cheese and that the compound is apparently formed from caprylic acid through the action of *Penicillium roqueforti* growing under conditions unfavorable for the normal growth of the mold. That flavor of Blue cheese which may be attributed to the methyl ketones appeared to be very pronounced in the cheese to which steapsin was added. Lane and Hammer (2) have observed that Blue cheese made from homogenized milk has a whiter appearance and that the mold has a more brilliant green color than the cheese made from unhomogenized milk. A similar difference between the "enzyme" and normal cheese was observed in this work, especially during the early stages of ripening. The "enzyme" cheeses could be identified solely by appearance owing to their chalky-whiteness and the brilliant green coloration of the mold. It has been noted at this station that the mold in Blue cheese made in the normal way loses much of its green color shortly after the cheese is placed in tin-foil. The green color of the mold reappears,

however, when the cheeses approach the desired degree of ripeness. This loss and reappearance of color of the mold was never observed in the "enzyme" cheese.

Cheese to which steapsin was added was marketed through the same channels as other cheese made at this station. It was accepted by the trade as evidenced by repeat sales and by the fact that very few comments were received concerning any difference in the cheese. Some persons commented that the flavor was "too strong." A slight bitterness was always detected by those more familiar with the cheese, resulting in a distinct preference on the part of these individuals for cheese made in the normal way.

Data showing the relative rates of fat hydrolysis and protein degradation in "enzyme" and normal cheeses are shown in Table 1. The cheeses

TABLE 1

Fat hydrolysis and protein degradation of 4.5 month old cheese from normal milk and from milk to which steapsin was added

Treatment of milk	Volatile acids		Amino nitrogen
	Soluble	Insoluble	
	ml.	*ml.*	*mg.*
Normal	26.8	2.4	17.3
Steapsin	54.6	13.8	13.8

selected for these analyses were from batches made in the same plant, from the same milk supply, on succeeding days. The volatile acids were determined according to the method described by Currie (1). The amino nitrogen determinations were made using the Van Slyke microgasometric method. Results for the volatile acids are expressed as milliliters of N/10 acid per 100 grams of cheese, and for amino nitrogen as milligrams of nitrogen per gram of fat and moisture-free cheese.

It is clearly shown that fat hydrolysis proceeded further in the cheese to which steapsin was added. The extent of protein degradation as indicated by the quantity of amino nitrogen in the cheese was probably not significantly different.

Several batches of cheese were made from pasteurized milk to which steapsin was added at the rate of two grams per 100 pounds of milk, but which were not inoculated with mold. These cheeses were handled in exactly the same way as the normal cheese except that they were not pierced. These cheeses had an intense rancid flavor when first examined after about one month. The rank flavor of butyric acid persisted to the virtual exclusion of all other flavors even up to the time the cheeses were over fifteen months old. The extent of fat hydrolysis in such cheese was as great or greater than in normal cheese or "steapsin" cheese inoculated with mold. Data on the soluble and insoluble volatile acids in these cheeses are shown in Table 2. Some idea of the volatile acids present may be gained by deter-

TABLE 2

Volatile acids in mold-free "steapsin" cheese

Lot no.	Age of cheese in months	Volatile acids in ml. N/10 acid per 100 gr. cheese		Barium value*
		Soluble	Insoluble	
1	8	80.1	8.0	
	12	111.5	12.7	41.54
2	3	72.6	2.7	
	12	96.8	11.1	41.06
3	7	97.2	16.8	
	11	96.8	13.85	41.38
4	7	105.2	16.0	
	11	107.9	16.1	41.37

* Determinations made by James Duncan, Assistant in Dairy Products, University of Minnesota.

mining the percentage of barium in the barium salt prepared from the volatile acids obtained on distillation, using the method described by Hammer and Sherwood (9). Barium values for the volatile acids obtained on distillation from mold-free "steapsin" cheese ranged from 41.06 to 41.54. These data also are shown in Table 2. Lane and Hammer (2) obtained barium values ranging from 40.42 to 42.00 for the volatile acids from normal Blue cheese 37 weeks of age. These results indicate that differences in the flavor of normal and mold-free "steapsin" cheese can not be ascribed to differences in the volatile acids in the cheese. The addition of steapsin accelerates the formation of the flavor constituents in the cheese but can not produce these flavors in the absence of the mold. These results are similar to those of Lane and Hammer (2) who found that in mold-free, unskewered cheese made from homogenized milk the flavor and aroma of methyl-n-amyl ketone could not be noted at any period during ripening.

SUMMARY

The ripening of Blue cheese may be accelerated by the addition of steapsin to the milk or to the curd. Approximately the same degree of flavor development was secured in five months in cheese to which commercial steapsin (Pfanstiehl) was added as in normal cheese in twelve months. While the "steapsin" cheese was accepted by the trade, competent judges considered it inferior to the normal cheese due to a characteristic bitter flavor.

Cheeses made with steapsin but which were not inoculated with mold became quite rancid and developed none of the typical flavor of Blue cheese. Although steapsin may accelerate the ripening of Blue cheese, it cannot produce the characteristic flavor of the cheese, unless *Penicillium roqueforti* is present and active.

REFERENCES

(1) CURRIE, JAMES N. Flavor of Roquefort cheese. J. Agr. Res. 2: 1–14. 1914.

(2) LANE, C. B., AND HAMMER, B. W. Bacteriology of cheese. III. Some factors affecting the ripening of Blue (Roquefort type) cheese. Iowa Agr. Exper. Sta. Res. Bull. 237. 1938.

(3) SHARP, P. F., AND DE TOMASI, J. A. Increase in non-lactic acidity in raw cream and its control. Proc. Intern. Assoc. Milk Dealers. (Lab. Sec.) 25: 3–20. 1932.

(4) ORLA-JENSEN. The action of certain proteolytic enzymes in the ripening of cheese. Ann. Agr. Suisse. 2: 95–100. 1901. Cited in Exper. Sta. Rec. 13: 387. 1901, 1902.

(5) WOJTKIEWICZ, A., AND INIKKOFF, G. The acceleration of the ripening of cheese. Tenth World's Dairy Congress. Milan. Section II: 335–339. 1934.

(6) FREEMAN, T. R., AND DAHLE, C. D. Rate of ripening in Cheddar cheese. Penn. Agr. Exper. Sta. Bull. 362. 1938.

(7) STÄRKLE, M. Die Methylketone im oxidativen Abbau einiger Triglyceride (bzw. Fettsäuren) durch Schimmelpilze unter Berücksichtigung der besonderen Ranzidität des Kokosfettes. Biochem. Zschr. 151: 371–415. 1924.

(8) HAMMER, B. W., AND BRYANT, H. W. A flavor constituent of Blue cheese (Roquefort type). Iowa State College J. Sci. 11: 281–285. 1937.

(9) HAMMER, B. W., AND SHERWOOD, F. F. The volatile acids produced by starters and by organisms isolated from them. Iowa Agr. Exper. Sta. Res. Bull. 80. 1923.

PRELIMINARY OBSERVATIONS ON THE TREATMENT OF PARCHMENT PAPER WITH SODIUM OR CALCIUM PROPIONATE[1]

H. MACY AND J. C. OLSON

Division of Dairy Husbandry, University of Minnesota

The protection of butter against surface molding has been an age-old problem. Many suggestions have been advanced as to methods for treating parchment wrappers and liners to destroy molds present on the paper or to inhibit subsequent growth of mold on the surface of butter or parchment. Numerous chemical compounds have been proposed and used but nearly all of them have had their disadvantages or have proved to be ineffective.[2]

Recently, sodium propionate and calcium propionate have been suggested as substances which might be effective inhibitors of mold growth when used for the treatment of parchment wrappers or liners. Consequently, preliminary trials have been run in our laboratories to determine the usefulness of these compounds[3] in the dairy industry.

EXPERIMENTAL METHODS

In these preliminary experiments, one-pound prints of commercial salted and unsalted butter were obtained and cut into eight equal parts. These portions were individually wrapped with pieces of parchment paper $4'' \times 5\frac{1}{2}''$ in size. Moisture and salt determinations were made on all lots of butter.

TRIAL I

Thirty-six eighth-pound prints of unsalted butter were placed upon a table in the laboratory and exposed to contamination from the air for 10 minutes so that five surfaces of each print were continuously exposed. Previous to, and during the exposure and wrapping, an electric fan was operated at full speed on the floor in the vicinity of the table. After exposure, the butter was immediately wrapped with parchment paper which had been immersed in 8, 16, or 20 per cent solutions (eight prints for each solution) of sodium propionate for a period of two minutes with twelve controls (three sets of four each, one set for each solution) wrapped in parchment immersed in sterile water. All the samples were placed in a humidor in which the atmosphere could be maintained at a humidity of approxi-

Received for publication January 16, 1939.

[1] Published with the approval of the Director as Paper 1678, Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] "Parchment Paper as a Source of Mold Infection in Butter," H. Macy and G. M. Pulkrabek. Minnesota Agricultural Experiment Station Bulletin 242. 1928.

[3] Sodium and calcium propionates were furnished through the courtesy of the E. I. Du Pont de Nemours & Company.

mately 100 per cent. The humidor was placed in a refrigerator for storage at 50° F. The samples were examined at the end of 2, 4, 6, and 12 weeks.

It may be noted in Table 1 that marked inhibition of mold growth on the

TABLE 1

Effect of treatment of parchment with aqueous solutions of sodium propionate on the growth of mold on unsalted and salted butter exposed to the air for 10 minutes before wrapping

Per cent of sodium propionate	Extent of growth at 50° F. after							
	2 weeks		4 weeks		6 weeks		12 weeks	
	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted
8	8+	8–	8++	8–	8+++	6– 1+	8+++	4– 3+
16	8–	8–	1– 7+	8–	7+	7–	7++	7–
20	8–	8–	3– 4+	8–	7+	8–	7++	8–
Controls*	12+++	12++	12++++	12++		12++		12++

Note: – = No visible growth.
+ = Scanty growth.
++ = Moderate growth.
+++ = Abundant growth.
++++ = Luxurious growth.

* Four controls for each lot of treated samples.

In the above and subsequent tables, the numbers followed by symbols may be interpreted for example as follows: 8+ means that all eight of the samples showed scanty growth; 1– 7+ means one sample showed no visible growth while seven showed scanty growth, etc.

Where less than eight samples are noted at any period, the remainder had been removed for analysis.

surface of the butter or parchment resulted from the use of sodium propionate in the various concentrations mentioned above. Visible mold did appear in two weeks time on the surface of the samples wrapped with parchment paper treated with an 8 per cent solution, although the amount of growth was very slight. No visible growth was observed at the end of two weeks on the prints wrapped with parchment treated with the 16 or 20 per cent solutions and in four cases none appeared even after four weeks of storage. The 16 and 20 per cent solutions still exhibited a restraining effect at the end of six weeks storage and at 12 weeks the molds had not developed as extensively as they had on the controls.

TRIAL II

In Trial II salted butter was subjected to the same conditions as described in Trial I. The moisture and salt in the butter were 15 per cent and 1.83 per cent respectively. The results of this trial are shown in Table 1. Complete inhibition of visible surface mold growth for a period of 12 weeks resulted when the parchments were treated with 16 per cent or 20 per cent solutions of sodium propionate. It is evident that the lower concentrations of sodium propionate were much more effective with the salted

butter than with the unsalted. Only one of the eight samples wrapped with parchment treated with an 8 per cent solution showed any definite mold growth at the end of the 6-weeks period, one colony being observed on that print, but at the end of 12 weeks only four of the remaining samples were free from visible mold.

In this trial, the controls showed moderate growth and appeared quite different from the unsalted controls. The mold colonies seemed to be quite numerous but were very small, about two millimeters in diameter. In contrast the unsalted controls in Trial I showed large, spreading colonies.

TRIAL III

In this experiment, thirty-six eighth-pound prints of unsalted butter were heavily seeded with a suspension of mold spores. The mold cultures were obtained from samples of moldy butter brought to the laboratory from a commercial creamery. The spores taken from plate cultures of these molds were dispersed in sterile water in an atomizer and then sprayed over the surface of the prints of butter. The samples were thereafter wrapped in untreated and treated parchment as described in Trial I.

The results are presented in Table 2. Visible surface mold was observed

TABLE 2

Effect of treatment of parchment with aqueous solutions of sodium propionate on the growth of mold on unsalted and salted butter sprayed with a mold spore suspension before wrapping

Per cent of sodium propionate	Extent of growth at 50° F. after							
	2 weeks		4 weeks		6 weeks		12 weeks	
	Un-Salted	Salted	Un-salted	Salted	Un-salted	Salted	Un-salted	Salted
8	8+++	8++	8+++	7++		6++		6++
16	8++	7– 1+	8+++	3– 4+		3– 3+		3– 3+
20	8–	8–	8++	8–	8+++	6–		6–
Controls	12++++	12++++						

Note: On all samples indicated as + growth, only one visible colony was observed.

after two weeks on samples wrapped in parchment treated with 8 and 16 per cent solutions of sodium propionate but some inhibition was noted in comparison with the controls. These prints were discarded at the end of the 4 weeks observation period because of the heavy mold growth. Complete inhibition of visible surface molding for a period of about three weeks resulted when the 20 per cent sodium propionate solution was used for treating the wrappers. This protection was not effective thereafter.

All controls showed a luxurious growth of surface mold and were discarded at the end of two weeks.

TRIAL IV

In this trial, salted butter was subjected to the same conditions as

those outlined in Trial III. The butter was from the same lot as that used in Trial II. As shown in Table 2, an 8 per cent sodium propionate solution was not as effective as 16 or 20 per cent solutions in protecting the butter or wrappers from molding.

Complete inhibition of visible surface mold was evident on the prints wrapped with parchment treated with a 20 per cent solution of sodium propionate, even after twelve weeks of storage at 50° F. The controls in all cases were covered with a luxurious growth of mold at the end of two weeks. The mold colonies were small but nevertheless were just as objectionable as the larger colonies observed on unsalted butter.

TRIAL V

Trial V, in general, duplicated Trial I except that calcium propionate was used instead of sodium propionate and the butter was exposed to the air for a period of thirty minutes rather than ten minutes. The results are shown in Table 3. Mold growth was inhibited for a period of two weeks in

TABLE 3

Effect of treatment of parchment with aqueous solutions of calcium propionate on th growth of mold on unsalted and salted butter exposed to the air for 30 minutes before wrapping

Per cent of calcium propionate	Extent of growth at 50° F. after							
	2 weeks		4 weeks		6 weeks		11 weeks	
	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted
8	8–	8–	7++	7+	7++	7+	7+++	7+
16	8–	8–	7+	1– 6+	7++	2– 5+	7+++	2– 5+
20	8–	8–	2– 5+	7–	7+	7–	7++	6– 1+
Controls	12+++	12++	12+++	12+++	12+++	12+++	12++++	12++++

all cases but at the end of four weeks all samples were moldy except two prints wrapped in parchment which had been treated with a 20 per cent solution of calcium propionate. After six weeks, all samples showed mold, but none were as bad as the controls. The highest concentration of propionate was clearly most effective.

In Trial VI salted butter was used and subjected to the same conditions as those described in Trial V. The moisture and salt in this butter were 16.3 per cent and 1.75 per cent respectively. The results of this trial are shown in Table 3. It would appear that in the case of the prints wrapped in the parchment treated with 8 per cent and 16 per cent solutions of calcium propionate, less inhibition resulted than when sodium propionate was used. (See Table 1.) However, the period of exposure and the salt concentration of the butter used in this trial were more apt to favor mold growth. The controls in Trial VI showed abundant and luxurious growth

whereas those in Trial II at no time during the 12 weeks showed more than moderate growth. Regardless of the differences in the composition and treatment of the butter, the results indicate a very marked inhibition when parchment treated with calcium propionate is used for salted butter.

TRIAL VII

In this experiment, conditions used in Trial III were duplicated. The results are shown in Table 4.

TABLE 4

Effect of treatment of parchment with aqueous solutions of calcium propionate on the growth of mold on unsalted and salted butter sprayed with a mold spore suspension before wrapping

Per cent of calcium propionate	Extent of growth at 50° F. after							
	2 weeks		4 weeks		6 weeks		11 weeks	
	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted	Unsalted	Salted
8	8++	8–	8++	4– 4+	8++	8+	8+++	8++
16	8–	8–	4– 4+	8–	3– 5+	8–	3– 5+	7–
20	8–	8–	4– 4++	8–	4– 4++	8–	4– 4++	8–
Controls	12++++	12+++	12++++	12+++	12++++	12+++	12++++	12++++

A very marked inhibition of surface mold growth was observed on the prints wrapped with parchment treated with each of the three solutions. The samples wrapped in parchment treated with 16 and 20 per cent solutions showed no visible mold growth at the end of two weeks, and after four weeks one-half of the samples still showed no visible growth. In the case of samples wrapped in parchment treated with a 20 per cent calcium propionate solution, one-half of the samples remained free of visible mold growth during eleven-weeks storage. The controls in contrast showed luxuriant surface growth.

In comparing Table 4 with Table 2, a rather marked difference can be observed in the relative inhibitory power of sodium and calcium propionate.

Representative samples were picked from this trial and photographs were taken to bring out more clearly the difference in appearance between the controls and the prints wrapped in treated parchment. Figure 1 shows (a) a control, (b) a print wrapped in parchment treated with a 16 per cent solution and (c) a print wrapped in parchment treated with a 20 per cent solution of calcium propionate. At the time this picture was taken the butter had been stored for a period of 13 weeks.

TRIAL VIII

Conditions in Trial VIII were the same as those in Trial VII except that salted butter was used. This butter was from the same lot as that used in

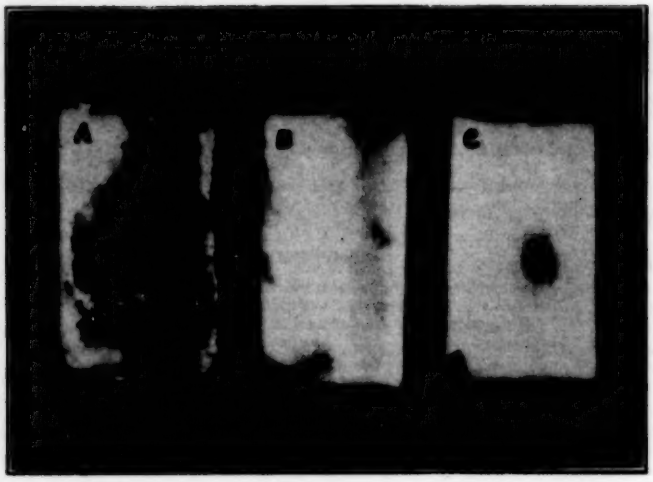

FIG. 1. This photograph shows the appearance of representative prints from Trial VII; A, a control, B, wrapper treated with 16 per cent solution, and C, wrapper treated with 20 per cent solution of calcium propionate. Thirteen weeks storage.

Trial III. Table 4 gives the results and again indicates the remarkable inhibitory power of calcium propionate.

Figure 2 shows three representative prints, (a) a control, (b) a print

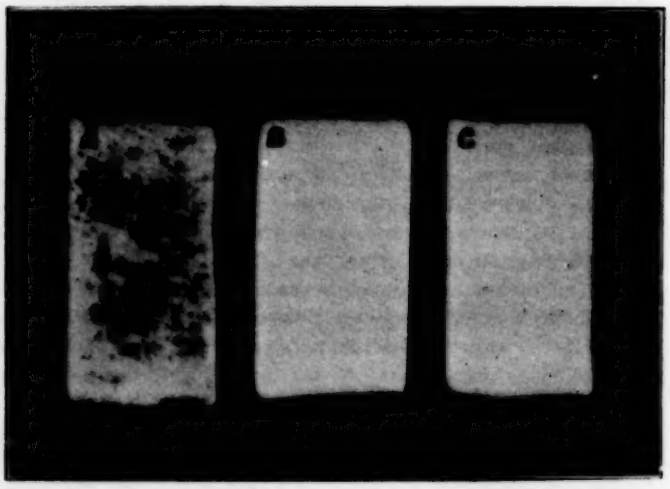

FIG. 2. This photograph shows the appearance of representative prints from Trial VIII; A, a control, B, wrapper treated with 16 per cent solution, and C, wrapper treated with 20 per cent solution of calcium propionate.

wrapped with parchment treated in a 16 per cent solution and (c) a print wrapped with parchment treated in a 20 per cent solution. Complete inhibition of visible surface mold throughout the duration of the storage period took place on samples wrapped with parchment treated with 16 and 20 per cent solutions of calcium propionate.

The controls as usual were covered with a luxuriant mold growth.

GENERAL DISCUSSION

In addition to the experiments previously described a number of other trials have been made using a mixture of sodium chloride and sodium propionate or calcium propionate. The results of these trials indicate that there might be some advantage in using a mixed solution of that sort for the treatment of parchment paper. In wrapping highly-salted butter, it may be advantageous to use a mixture of sodium chloride and one of the propionates for inhibiting molds as well as to prevent surface mottling.

In order to determine the effectiveness of the various concentrations of the propionates when the wrappers were merely dipped rather than soaked in the solutions, a duplication of trials I, II, V and VI was made. The results were fairly comparable with those obtained by immersing the parchment for two minutes except that they were more variable.

The ability of the sodium and calcium propionate solutions to inhibit mold growth when used in the treatment of butter wrappers is quite significant. The exact nature of the inhibitory principle has not as yet been determined.

When mold spots appeared on the surface of the butter the parchment paper was usually somewhat smudged where it was in contact with the moldy area. In most instances, the mold appeared to develop first on the butter.

In these preliminary trials, the butter and parchment were subjected to very extreme conditions regarding initial mold contamination and subsequent storage. The heavy seeding of the butter with mold spores from the air or by spraying with suspensions from cultures, was far more severe than that which butter would encounter in the ordinary manufacturing process or subsequent handling. Even under such conditions actual prevention of visible surface mold growth for a period of twelve weeks was observed in certain of the trials. Controls which were subjected to the same treatment except for the fact that they were wrapped with parchment immersed in sterile water, were covered with abundant growth in two weeks time.

During periods of observation it was noticed that the controls, particularly the unsalted butter, exhibited a decidedly cheesy flavor. This was not found to be true of prints wrapped with some of the treated parchments. Further studies on this point are also in progress.

The treated parchment did not appear to contribute any appreciable odor to the butter. Immediately after immersion the parchment dipped in calcium propionate solutions was quite bitter and the surface of the butter in contact with it was slightly bitter at first but soon lost such a taste. The sodium propionate solutions were somewhat sweet but did not seem to contribute any noticeable flavor to the surface of the butter.

Specific recommendations as to the proper concentrations of solutions to be used cannot be made at this time. Further trials are now in progress to determine the most practical and efficient methods which may be used for satisfactory inhibition of surface mold growth.

SUMMARY

1. Unsalted and salted butters deliberately contaminated with mold spores were wrapped in parchment papers, soaked either in sterile distilled water, or 8, 16 and 20 per cent solutions of sodium or calcium propionate, and stored for 11–12 weeks at high humidities at 50° F. to observe the extent of mold growth on the surface of the butter or parchment.

2. Marked inhibition of mold development occurred when the butters were wrapped in parchment papers treated with the solutions of sodium or calcium propionate, especially with the higher concentrations.

3. The inhibition was much more pronounced in the case of salted butter.

COMPARISONS OF THE INACTIVATION OF PHOSPHATASE ENZYME AND THE KILLING OF *ESCHERICHIA-AEROBACTER* ORGANISMS AS CRITERIA OF SATISFACTORY PASTEURIZATION OF ICE CREAM MIX[1]

F. E. NELSON, W. J. CAULFIELD, AND W. H. MARTIN
Kansas Agricultural Experiment Station

In a previous study (1) it was established that ice cream mix pasteurized at 150° F. (65.6° C.) may show a negative phosphatase test after an exposure of only 15 minutes, and in no case was a positive test observed after a 25 minute exposure. Mix held at 160° F. (71.1° C.) for 5 minutes invariably was negative to the phosphatase test.

The question arises whether mix pasteurized sufficiently to yield a negative phosphatase test is also adequately pasteurized with respect to the *Escherichia-Aerobacter* group of bacteria. Since both the phosphatase test and the determination of the presence of *Escherichia-Aerobacter* organisms are advocated as measures of the adequacy of pasteurization, the relationship between the two should be established. The protective action of the solids of the ice cream mix might not be the same for the enzyme and the organism.

The use of the two determinations already has been recommended for market milk (2, 3). A sample negative to the phosphatase test and containing organisms of the *Escherichia-Aerobacter* group is considered by many investigators to have been contaminated following satisfactory pasteurization. Such use of the two tests has proved of value to the writers in determining contamination of ice cream mix under plant conditions. However, the general applicability of the procedure to ice cream can be determined only by comparison of the two tests, using samples obtained under controlled conditions.

METHODS

The ice cream mix used was calculated to contain 12 per cent fat, 10 per cent serum solids, 15 per cent sugar, and 0.35 of one per cent gelatin. Ingredients used in preparing the mix included raw cream, raw skim milk, condensed skim milk, sugar and gelatin. Cultures of *Escherichia-Aerobacter* organisms grown in sterile milk for 24 hours at 37° C. and then held at 4° C. for 16 hours were added to all except the uninoculated control mixes in appropriate quantities immediately before pasteurization exposures were begun. The cultures used were the most heat-resistant of eighty-eight strains previ-

Received for publication January 18, 1939.

[1] Contribution No. 176, Department of Bacteriology and No. 128, Department of Dairy Husbandry.

ously isolated from ice cream and were, with one exception, able to withstand 143.5° F. (61.9° C.) for 30 minutes when heated in ice cream mix of the composition used in these experiments. Laboratory pasteurization was made by carefully placing about 10 ml. of mix in the bottom of a sterile 18 × 150 mm. test tube which was closed by a sterile stopper and immersing the tube in a stirred water bath of the desired temperature to such a depth that the water reached at least two inches above the surface of the mix. In semi-commercial pasteurization the mix was heated in a 10 gallon steam-jacketed copper kettle equipped with a mechanical agitator, but with no provisions for the prevention of splattering or for insuring adequate heating of the foam. The samples were iced immediately after pasteurization. Phosphatase tests were made by the modified New York City field test devised by Scharer (4, 5) in all cases and also by the laboratory test of Gilcreas and Davis (6) in most cases. The numbers of *Escherichia-Aerobacter* organisms were determined by inoculations into triplicate tubes of brilliant green lactose peptone bile 2 per cent broth according to the tentative method outlined by the American Public Health Association (7). The most probable number of organisms per milliliter was determined from McCrady's tables of probability (8), with the exception of one series in which the numbers of *Escherichia-Aerobacter* organisms in the unpasteurized mixes were calculated from the microscopic counts of the organisms present in the cultures added.

EXPERIMENTAL RESULTS

The results of the first and second series of laboratory pasteurizations are shown in Tables 1 and 2, respectively. The *Escherichia-Aerobacter* organisms used did not survive in a sample of ice cream mix which was negative to the field or the laboratory test for the phosphatase enzyme, except in the case of the uninoculated control heated to 160° F. (71.1° C.) for 5 minutes in the second series (Table 2). Since only one of the triplicate tubes was positive in this case, the result was probably due to some error in technique. Except for this one instance, there was at least a slight residuum of active phosphatase present after all of the test organisms had been killed. In some cases a considerable amount of the enzyme remained active after death of the test organisms. A temperature of 145° F. (62.8° C.) for 30 minutes never inactivated all of the phosphatase and on some occasions failed to kill all of the *Escherichia-Aerobacter* organisms present in the mix. Exposure to 150° F. (65.6° C.) for 20 minutes destroyed all of the test organisms and gave a negative value for the New York City field test with but two exceptions, both in the first series. With but one exception, this same treatment reduced the phenol value of the samples, as measured with the Gilcreas and Davis test, to below 0.04 mg. of phenol per 0.5 ml. sample, the standard set by Gilcreas and Davis as the maximum permissible in a sample of market milk that

TABLE 1

Comparisons of phosphatase inactivation and destruction of Escherichia-Aerobacter organisms during laboratory pasteurizations of ice cream mix

Treatment of sample		Uninoculated control		Organism 107		Organism 11–1		Organism 116		Organism 15–1		Organism 32 C	
Temp. (°F.)	Time (min.)	Probable no. of *E.*-*A.* organisms per ml.[1]	Phos. test[2]	Probable no. of *E.*-*A.* organisms per ml.	Phos. test	Probable no. of *E.*-*A.* organisms per ml.	Phos test	Probable no. of *E.*-*A.* organisms per ml.	Phos. test	Probable no. of *E.*-*A.* organisms per ml.	Phos. test	Probable no. of *E.*-*A.* organisms per ml.	Phos. test
Control	0	> 140	+++	> 14,000	+++	4,500	+++	11,000	+++	11,000	+++	4,500	+++
145	20	0	++	0.4	++	0	++	0	++	0	++	0.3	++
	30	0	+	1.4	+	0	+	0	+	0.3	+	0	+
150	10	0	+BA	0	+BA	0	+BA	0	+BA	0	+BA	0	+BA
	15	0	+BA	0	+BA	0	+BA	0	+BA	0	+BA	0	–
	20	0	–	0	–	0	±	0	–	0	±	0	–
	30	0	–	0	–	0	–	0	–	0	–	0	–
160	5	0	–	0	–	0	–	0	–	0	–	0	–
	10	0	–	0	–	0	–	0	–	0	–	0	–
	30	0	–	0	–	0	–	0	–	0	–	0	–

[1] From McCrady's tables.

[2] New York City field test results (+BA = + only when butyl alcohol was used in reading test).

TABLE 2

Further comparisons of phosphatase inactivation and destruction of Escherichia-Aerobacter organisms during laboratory pasteurization of ice cream mix

Treatment of sample		Uninoculated control			Organism 60 gc			Organism 145			Organism 154			Organism 158 g2		
Temp. (°F.)	Time (min.)	Probable no. of *E.-A.* organisms per ml.[1]	Phos. test		Probable no. of *E.-A.* organisms per ml.	Phos. test		Probable no. of *E.-A.* organisms per ml.	Phos. test		Probable no. of *E.-A.* organisms per ml.	Phos. test		Probable no. of *E.-A.* organisms per ml.	Phos. test	
			NYC[2]	GD[3]		NYC	GD		NYC	GD		NYC	GD		NYC	GD
Control	0	> 14,000	+++	X	>140,000	+++	X	45,000	+++	> 0.15	45,000	+++	> 0.15	> 140,000	+++	> 0.15
145	20	0	++	X	0	++	X	0	++	> 0.15	0	++	> 0.15	0	++	> 0.15
	30	0	+	X	0	+	X	0	+	> 0.15	0	+	> 0.15	0	+	> 0.15
150	10	0	+ BA	0.15	0	+ BA	0.12	0	+ BA	0.09	0	+	0.15	0	+	0.15
	15	0	+ BA	0.05	0	+ BA	0.05	0	+ BA	0.04	0	+ BA	0.09	0	+ BA	0.09
	20	0	–	0.04	0	–	0.035	0	–	0.03	0	–	0.035	0	–	0.035
	30	0	–	0.025	0	–	0.015	0	–	0.02	0	–	0.02	0	–	0.015
160	5	0.4	–	0.02	0	–	0.015	0	–	0.015	0	–	0.01	0	–	0.03
	10	0	–	0.015	0	–	0.015	0	–	0.015	0	–	0.015	0	–	0.015
	30	0	–	0.015	0	–	0.01	0	–	0.01	0	–	0.01	0	–	0.01

[1] From McCrady's tables.
[2] New York City field test results (+ BA = + only when butyl alcohol was used in reading test).
[3] Gilcreas and Davis test (phenol values).
X = no test made.

TABLE 3

Comparisons of phosphatase inactivation and destruction of Escherichia-Aerobacter organisms in ice cream mix pasteurized on a semi-commercial scale

Treatment of sample		Organism 11–1			Organism B–2–c			Organism 107			Organism 15–1			Organism 116		
Temp. (°F.)	Time (min.)	Probable no. *E.-A.* organisms per ml.[1]	Phosphatase test		Probable no. *E.-A.* organisms per ml.	Phosphatase test		Probable no. *E.-A.* organisms per ml.	Phosphatase test		Probable no. *E.-A.* organisms per ml.	Phosphatase test		Probable no. *E.-A.* organisms per ml.	Phosphatase test	
			NYC[2]	GD[3]		NYC	GD		NYC	GD		NYC	GD		NYC	GD
Control	0	18,000	+++	> 0.15	120,000	+++	> 0.15	56,000	+++	> 0.15	18,000	+++	> 0.15	14,000	+++	> 0.15
145	0	> 1,400	+++	> 0.15	> 1,400	+++	> 0.15	> 1 400	+++	> 0.15	> 1,400	+++	> 0.15	450	+++	> 0.15
	20	0.4	+	> 0.15	0.4	+	> 0.15	1.5	+	> 0.15	2.5	+	> 0.15	2.5	+	> 0.15
	30	25.0	+	> 0.15	0.4	+	> 0.15	2.5	+	> 0.15	4.5	+	> 0.15	2.5	+	> 0.15
150	0	> 1,400	++	> 0.15	> 1,400	++	> 0.15	> 1,400	++	> 0.15	> 1,400	+++	> 0.15	450	+++	> 0.15
	10	4.5	–	0.035	0.7	+s[4]	0.14	2.5	+s	0.15	9.5	+s	0.09	0.4	+s	0.15
	15	0.9	–	0.02	2.5	–	0.04	2.5	–	0.06	2.5	–	0.05	2.0	+s	0.04
	20	0.9	–	0.015	2.0	–	0.035	9.5	–	0.04	9.5	–	0.03	3.0	–	0.015
	30	0	–	0.015	2.5	–	0.01	0.9	–	0.03	2.5	–	0.01	2.5	–	0.01
160	0	4.5	–	0.01	2.5	+	> 0.15	25.0	+	> 0.15	2.5	+	> 0.15	4.5	+	> 0.15
	5	0.4	–	0.01	2.5	–	0.01	4.5	–	0.03	2.5	–	0.01	9.5	–	0.01
	10	0.7	–	0.01	2.5	–	0.01	2.5	–	0.01	4.5	–	0.01	2.5	–	0.01
	30	0.4	–	0.01	0.9	–	0.01	2.5	–	0.01	2.5	–	0.01	2.5	–	0.01

[1] From McCrady's tables.
[2] New York City field test (no butyl alcohol used).
[3] Gilcreas and Davis test (phenol values).
[4] +s = slightly positive.

had been adequately pasteurized. This value has been used arbitrarily in evaluating the results obtained in this study. At 160° F. (71.1° C.), 5 minutes exposure was sufficient to inactivate the phosphatase in all cases and to kill all of the test organisms, except in the one case previously mentioned.

The results of pasteurizations on a semi-commercial scale are given in Table 3. Although the results of the inactivation of phosphatase, as judged by the Gilcreas and Davis test, are in close agreement with those obtained in the laboratory pasteurization trials, the results obtained with the field test are not so satisfactory, probably due to the border-line cases which were missed because butyl alcohol was not used to concentrate the color. This series of tests was run before the butyl alcohol modification had been introduced. The presence of organisms of the *Escherichia-Aerobacter* group in all samples except one, irrespective of the heat treatment given, would indicate that contamination of the mix was occurring during or immediately following pasteurization. This is further emphasized by comparison with the results of the laboratory pasteurizations in which the same organisms were used. Contamination could easily have occurred, since no provisions were made for heating the foam and for prevention of splattering and drying on the sides of the container. In most cases, the numbers of organisms found in the pasteurized mix could be attributed to contamination of the 60 pound batch by as little as approximately one milliliter of the original heavily inoculated mix. Such a source is indicated by the lack of correlation between time of holding in each temperature series and the number of organisms surviving. The addition of such small quantities of incompletely pasteurized mix (approximately 0.04 of one per cent of the total volume) would be too small to be detected by any of the phosphatase tests now being used.

DISCUSSION

The results would indicate that in ice cream mix, the milk enzyme phosphatase has greater resistance to heat than have the more heat-resistant members of the *Escherichia-Aerobacter* group of organisms. More resistant strains than those used in these studies might possibly change the observed difference between enzyme inactivation and death of the organisms, but such strains do not occur commonly. Therefore, if organisms of this group are present in a sample negative to the phosphatase test, contamination is probable. That such contamination may occur during the pasteurization procedure is indicated by the results obtained in pasteurization on a semi-commercial scale as compared with laboratory pasteurization under controlled conditions.

The ease with which these two determinations can be made and the definiteness of the results obtained with each make them ideal for use in dairy control work, both in the plant and in the public health laboratory. By

using the two determinations together, the diagnostic value of each is increased considerably. The difficulty of keeping ice cream equipment, particularly the viscolizer or homogenizer, in proper sanitary condition makes a reliable means of demonstrating contamination of considerable value.

CONCLUSIONS

1. Using ice cream mix as the substratum, the milk enzyme phosphatase is more resistant to heat than are the more heat-resistant bacteria of the *Escherichia-Aerobacter* group.

2. The presence of bacteria of the *Escherichia-Aerobacter* group in ice cream mix or ice cream which is negative to the phosphatase test can be considered an indication of contamination during or following pasteurization.

REFERENCES

(1) CAULFIELD, W. J., AND MARTIN, W. H. Certain factors affecting the phosphatase test when applied to ice cream. J. DAIRY SC. 22: 261–270. 1939.

(2) TIEDEMAN, W. VON D., AND HOHL, N. J. Improved laboratory control of pasteurized milk. Am. J. Pub. Health 28: 629–632. 1938.

(3) GUNSALUS, I. C., AND STARK, C. N. Formate ricinoleate and brilliant green bile broths to detect coliform organisms in pasteurized milk. Am. J. Pub. Health 28: 832–834. 1938.

(4) SCHARER, H. A. Rapid phosphomonoesterase test for control of dairy pasteurization. J. DAIRY SC. 21: 21–34. 1938.

(5) SCHARER, H. Improvements in the rapid phosphatase test for detection of improper pasteurization of milk and its products. J. Milk Techn. 1: 35–38. 1938.

(6) GILCREAS, F. W., AND DAVIS, W. S. The practical value of the phosphatase test in determining the efficiency of pasteurization. Proc. Int. Assoc. Milk Dealers, Lab. Sect. p. 34–56. 1937.

(7) AMERICAN PUBLIC HEALTH ASSOCIATION. Standard Methods of Milk Analysis, 6th ed. New York. 1934.

(8) BUCHANAN, R. E., AND FULMER, E. I. Physiology and Biochemistry of Bacteria, Vol. I, p. 11. Williams and Wilkins Co., Baltimore. 1928.

STUDY OF PHYSICAL AND CHEMICAL PROPERTIES OF THE ADSORPTION "MEMBRANE" AROUND THE FAT GLOBULES IN CREAM. I. THE EFFECT OF THE ADSORPTION "MEMBRANE" OF SYNTHETIC CREAMS ON CURD TENSION OF COW'S MILK*

N. P. TARASSUK† AND L. S. PALMER

Division of Agricultural Biochemistry, University of Minnesota, St. Paul

The presence of an adsorption "membrane" around the fat globules in cow's milk has been demonstrated by the work of Storch (1), Völtz (2), Palmer and Samuelsson (3), Hattori (4), Palmer and Wiese (5), Schwarz and Fischer (6), and others. Palmer and Wiese (5), Wiese and Palmer (7), Rimpila and Palmer (8), have shown beyond any doubt that the adsorption layer consists of protein, phospholipides and other lipide substances. These investigations have proved that the protein of the "membrane" is different from any of the previously known proteins of milk, which is in agreement with the work of Hattori (4) and has been substantiated by Schwarz and Fischer (6).

While a considerable amount of work has been done on the methods of isolation of the "membrane" material, on its composition and the chemical nature of its constituents, much remains to be learned regarding its properties in relation to milk and milk products.

Palmer and Tarassuk (9) have shown that the "membrane" when released in buttermilk by churning sweet cream, lowers the curd tension. They have also shown that the artificial fat globule adsorption "membrane" derived by emulsifying butter fat in normal rennet whey likewise reduces the curd tension of milk plasma when released by churning but the adsorption "membrane" derived from skim milk does not possess this curd tension reducing property. Since the adsorption "membrane" derived from whey is very low in phospholipide content, as shown by Rimpila and Palmer (8), Palmer and Tarassuk suggested that the protein component of the fat globule "membrane" is the major factor concerned in the curd tension reducing effect of the "membrane."

The present study deals with the elucidation of the question as to which constituent, or constituents, of the adsorption "membrane"—the phospholipide, protein, or phospholipide-protein complex—is, or are, involved in the

Received for publication January 25, 1939.

* This paper represents a portion of the thesis presented by N. P. Tarassuk in partial fulfillment of the requirements for the degree of Ph.D., University of Minnesota, 1937. Published with the approval of the Director as Paper No. 1685, Journal Series, Minnesota Agricultural Experiment Station.

† Now at University of California, Dairy Industry Division, Davis, California.

reduction of curd tension, as well as with the exposition of some other changes in the properties of buttermilks which were obtained from various synthetic creams whose fat globules adsorption "membrane" was derived from the material of known and predetermined chemical composition.

EXPERIMENTAL

General procedure and methods employed. Synthetic creams were made by emulsifying[1] fresh, pure, melted butter fat in an aqueous sol of the desired emulsifier to give the degree of dispersion of fat globules similar to that of natural cream, and to contain 27–30 per cent fat. The temperature of emulsification was 37° C. The cream emulsion was allowed to stand for two to three hours in ice water, observed for stability and warmed again to 37° C. at which time a sufficient amount of fresh raw skim milk was added to make 3.5–4.0 per cent "remade" whole milk. "Remade" whole was then separated to give "remade" cream and "remade" skim. "Remade" cream on standing (usually overnight) at 8°–10° C. was churned in a glass Dazey churn to give "remade" buttermilk.

Curd tension was determined by a modification of Hill's[2] method as described by Morris and Richardson (10), the only difference being that the temperature used was 35° C. instead of 37° C. Generally, rennet extract was used as a coagulant, but in some experiments, for definite purposes, other coagulants, such as rennet + $CaCl_2$, pepsin, pepsin + $CaCl_2$, and papain, were used.

Surface tension was determined at 20°–21° C. by means of the Cenco-du-Noüy tensiometer.

Casein, albumin, and globulin nitrogen was determined by Moir's (11) method, and total nitrogen either by the usual macro-Kjeldahl-Arnold method or by Cavett's (12) improved micro-Kjeldahl method.

Calcium and phosphorus were determined by the method of Morris, Nelson and Palmer (13). Butter fat was determined by the Babcock procedure using the Minnesota reagent except when otherwise indicated. Total solids were determined by the Mojonnier method.

Hydrogen ion concentrations were determined electrometrically using the Bailey hydrogen electrode.

Special experimental procedures will be described in connection with the several experiments.

EXPERIMENTAL RESULTS

Palmer and Tarassuk (9) have shown that when pure butter fat is emulsified in fresh rennet whey, the fat globule adsorption "membrane" so

[1] The material was passed four times through a two-quart two-cylinder Club Aluminum Products Co. hand "homogenizer."

[2] The method is essentially the standard Hill test except for the older curd knives (diam. 2 inch) and the substitution of one ml. of rennet extract as coagulant for pepsin + $CaCl_2$.

formed may have a greater reducing effect on the curd tension of buttermilk than the natural "membrane." This suggested the possibility of employing sols made from dried whey for the emulsification of butter fat in order to find out the nature of the adsorbed substance responsible for the lowering of curd tension.

The effect of adsorption "membrane" derived from dried whey on curd tension of buttermilk. Two kinds of dried whey were used as the source of the adsorption "membrane," dried acid whey and dried rennet whey.[3] Since the results were identical in both cases we shall present only the data on dried rennet whey cream and "remade" milks derived from it.

The dried whey had the following composition:

Lactose	75.58%	Ca	0.54%
Protein	14.50	P	0.52
Ash	6.80	H_2O	2.32
Fat	0.80		

The dried whey dispersed readily in water. An aqueous sol of the powder having the same or slightly higher concentration of total solids than in normal rennet whey was used as the emulsifying agent for pure, dry butter fat. The pH of this sol in experiment (A) was 6.45 and in experiment (C)—6.35. In experiments (A) and (C) mixed herd skim milk was used to make "remade" whole milk and in experiment (B) skim milk high in total solids (Guernsey milk) was used for the same purpose.

In their stability and microscopic appearance the whey-powder creams resembled very closely natural cream. There was no separation of phases even on standing for three days.

As can be seen from Table 1, the curd tension values of "remade" buttermilk in all experiments are strikingly low as compared with the respective "remade" whole and "remade" skim milks.

It is well known that a high positive correlation exists between the percentage of solids-not-fat of milk and its curd tension. Tarassuk and Richardson (14) reported a correlation coefficient of $+0.85 \pm 0.3$, calculated from the data on fifty samples of milk from different cows. The amount of casein seems to be the most important solids-not-fat component determining the curd tension of milk (14) (15). Espe and Dye (16), Doan and Welch (17), and many others, have reported that the curd tension of normal milk is almost a linear function of the casein content.

The "remade" milks in Table 1 represent a wide variation in percentage of solids-not-fat and the relationship between the solids-not-fat and the curd tension holds very well in case of all "remade" milks with the exception of the "remade" buttermilks. It is true that the percentage of casein in the "remade" buttermilk is slightly lower than in the "remade" skim milks but

[3] The dried wheys were experimental spray process products furnished by Abbotts Dairies, Inc., Cameron, Wisconsin.

this fact cannot account for the extremely low curd tension of the "remade" buttermilks. Thus, in (A), the difference between the percentage casein in the "remade" skim and the "remade" buttermilk is 0.14, yet the curd tensions are respectively 50 and zero. The "remade" buttermilk in this case gave only a slightly visible coagulation and did not clot. There was no syneresis (separation of whey).

TABLE 1

Curd tension and composition of various milks involving the use of synthetic cream made by emulsifying butter fat in dried rennet whey sol

Product tested	Curd tension	pH	Fat	Total solids	Solids-not-fat	Protein analysis[1]		
						Casein	Albumin-globulin	Non-prot. N
	gms.		%	%	%	%	%	%
Cream (A):								
Original skim	67	.....	0.04	9.20	9.16	2.36	0.69	0.023
"Remade" whole	38	.....	3.90	.....	.....	.....	.....	.....
"Remade" skim	50	6.57	0.40	9.10	8.70	2.06	0.69	0.041
"Remade" buttermilk	0	6.12	2.75	10.68	7.93	1.92	0.43	0.045
Cream (B):								
Original skim	145	6.51	0.04	10.64	10.60	.....	.....	.....
"Remade" whole	112	6.33	3.00	13.80	10.80	.....	.....	.....
"Remade" skim	121	6.44	0.08	10.70	10.62	3.64	0.59	0.005
"Remade" buttermilk[2]	48	5.78	1.25	12.22	11.97	3.52	0.66	0.005
Cream (C):								
Original skim	75	6.60	.....	.....	.....	.....	.....	.....
"Remade" skim	60	6.60	0.11	9.28	9.17	2.44	.....	.....
"Remade" whole	45	6.48	3.60	12.66	9.06	2.38	.....	.....
"Remade" buttermilk	7	6.12	2.20	10.80	8.60	1.99	.....	.....

[1] Percentage of casein, albumin and globulin was calculated by multiplying the percentage of nitrogen of the respective proteins by the factor 6.35. Non-protein N was determined by subtracting casein N and albumin-globulin N from total N.

[2] Curd tension of natural buttermilk obtained from the same whole milk as the skim and fat used in (B)—104.

A portion of the same "remade" buttermilk was pervaporated at room temperature to contain 22.84 per cent of total solids, 6.50 per cent of butter fat and 16.34 per cent of solids-not-fat. Normally, a milk with ten per cent solids-not-fat, or higher, has a curd tension above 100 grams (see Table 1 (B)); yet, this "remade" buttermilk with 16.34 per cent of solids-not-fat gave a curd tension of only 41 grams.

The data presented in Table 2 give additional and conclusive evidence of the futility of attempting to explain the low curd tension of the "remade" buttermilks by their content of solids-not-fat or of casein. It will also be noted from this table that the direct addition of an aqueous sol of dried whey to milk has no other effect on its curd tension except that of dilution.

TABLE 2

Curd tension and composition of "remade" buttermilk as compared to original skim and "remade" whole which have been diluted with water or aqueous sol of dried whey

Product tested	Curd tension	Fat	Total solids	Solids-not-fat	Casein
	gms.	%	%	%	%
Cream (A):					
Original skim	67	0.040	9.20	9.160	2.36
Original skim diluted 10% with H_2O	52	0.036	8.28	8.244	2.12
Original skim diluted 20% with H_2O	48	0.032	7.36	7.328	1.89
Original skim diluted 10% with aqueous whey powder sol	52	0.036	8.28	8.244	2.12
"Remade" buttermilk	*0*	*2.75*	*10.68*	*7.93*	*1.92*
Cream (C):					
"Remade" whole	45	3.60	12.66	9.06	2.38
"Remade" whole diluted 30% with H_2O	29	2.52	8.86	6.34	1.67
"Remade" buttermilk	*7*	*2.20*	*10.80*	*8.60*	*1.99*

The extremely low curd tension of these "remade" buttermilks becomes even more significant when we consider their pH. It can be seen from Table 1 that the "remade" buttermilks invariably had a much lower pH than their respective "remade" whole or skim milk. This fact will be treated in detail in the paper to follow; at the present it will suffice to point out that the curd tension of milk normally increases with drop in pH (14) (15). The peculiar relation of pH to curd tension of the "remade" buttermilks can be better appreciated from the fact that when the pH of the "remade" buttermilk of cream (B) (see Table 1) was increased from the initial 5.78 to 6.24 by adding 7 ml. of 0.2 N NaOH to 125 ml. of buttermilk, the curd tension was lowered from the initial 48 grams to 10 grams. At the same time the "remade" skim obtained from the same cream had a pH value of 6.44 and a curd tension of 121 grams, thus giving a difference of 111 grams between the curd tensions of "remade" skim and "remade" buttermilk.

A factor which could possibly account, at least partially, for the low curd tension of "remade" buttermilk is the degree of dispersion of butter fat in the buttermilk. Homogenization of whole milk reduces its curd tension, and it is generally believed that this is due to the effect of homogenization on the fat phase. Chambers (18), in his paper dealing with soft curd milk produced by intense sonic vibration, found, that, "the subdivision of as little as 0.25% of butter fat in a sample of skim milk brought about as great a reduction in tension as was accomplished by the vibrator on whole milk."

Microscopic examination of the "remade" buttermilk revealed that it contained a greater number of finely dispersed fat globules than its "remade"

whole or "remade" skim milk. To determine the extent of the possible effect of the presence of these finely divided fat globules on the curd tension of "remade" buttermilk the following experiments were performed:

(a) *Partial removal of fat.* "Remade" buttermilk and "remade" skim from cream (B) (see Table 1) were warmed to 37° C. and centrifuged for 20 min. at 1800 r.p.m., using an International Equipment Co. size No. 2 centrifuge. The lower layer, poorer in fat, was pipetted out and the curd tension of the samples determined. The results are given in Table 3.

TABLE 3

Effect of partial removal of fat on curd tension of "remade" skim and "remade" buttermilk

Treatment of sample	Butter fat	Curd tension
	%	*gms.*
"Remade" skim (control)	0.08	121
"Remade" skim (centrifuged)	< 0.02	122
"Remade" buttermilk (control)	1.25	48
"Remade" buttermilk (centrifuged)	0.24	52

(b) *Addition of homogenized cream to skim milk.* Synthetic cream prepared from butter fat and sol of dried rennet whey, and natural cream, both containing 21.5 per cent of butter fat, were warmed to 110° F. and homogenized by using the single stage of a Gaulin homogenizer. The pressure applied was 2000 lbs. for the synthetic cream and 3000 lbs. for the natural cream.

In experiment I, a sufficient amount of each homogenized cream was added to the "remade" skim of cream (B) (see Table 1) and the curd

TABLE 4

Effect of degree of dispersion of butter fat on curd tension of milk

Exp.	Treatment of sample	Butter fat	Curd tension
		%	*gms.*
I	"Remade" skim	0.08	119
	"Remade" buttermilk	1.25	50
	90 ml. of "remade" skim + 10 ml. of 21.5% natural homogenized cream	> 2.0[1]	94
	90 ml. of "remade" skim + 10 ml. of 21.5% synthetic homogenized cream	> 2.0[1]	100
	Control: 90 ml. of "remade" skim + 10 ml. of natural non-homogenized cream	> 2.0[1]	100
II	Natural skim	0.04	74
	90 ml. of skim + 10 ml. of natural homogenized cream	> 2.0[1]	60
	90 ml. of skim + 10 ml. of synthetic homogenized cream	> 2.0[1]	64
	90 ml. of skim + 10 ml. of water	—	65

[1] Per cent of fat is estimated from the amount of cream added.

tension determined after a thorough distribution of the homogenized cream in the "remade" skim milk. The result was compared with the curd tension of the "remade" buttermilk from the same cream. In experiment II, the homogenized creams were added to natural skim milk. The data in Table 4 show that the presence of finely divided butter fat globules in the "remade" buttermilk can account for very little, if any, of its lost curd tension.

The effect of the adsorption "membrane" derived from the materials other than dried whey on the curd tension of buttermilk. Other materials used as the source of adsorption "membrane" for making synthetic creams were: skim milk powder, calcium caseinate, commercial gelatin, purified (isoelectric) gelatin, and tissue fibrinogen. The general procedure employed in making the synthetic creams and "remade" milks has been described above. In all cases the skim milk used for making "remade" whole milk from synthetic cream was fresh raw mixed herd skim milk. Other data pertinent to the synthetic creams and their "remade" milks are as follows:

(a) *Skim milk powder cream.* Spray process skim milk powder, easily dispersable in water, was used. An aqueous sol, containing 9 per cent total solids, served as the emulsifying agent. The fat emulsion appeared to be very stable and there was no separation on standing in ice water for 3.5 hours. The churning time of the "remade" cream was 50 minutes.

(b) *Calcium caseinate cream.* The emulsifying agent was a 3 per cent calcium caseinate sol.[4] The pH of the sol was 7.31. The cream emulsion was stable and the churning of the "remade" cream took 40 minutes.

(c) *Gelatin cream.* Several separate experiments were conducted using gelatin as the sole source of adsorption "membrane." In experiments I and II a commercial gelatin and in experiment III a purified (isoelectric) gelatin was used. The isoelectric gelatin contained 0.05 per cent ash and 16.03 per cent nitrogen. The isoelectric gelatin being pure protein removed any possibility that the impurities usually present in commercial gelatin could be of significance in the curd tension tests. In all cases the emulsifying agent was a 0.5 per cent gelatin sol. The pH of the commercial gelatin sol was 5.42 and that of the isoelectric gelatin was 4.63. The gelatin creams were very stable and there was no separation of phases on standing at 7° C. for as long as 27 hours. The churnability of the gelatin "remade" creams was quite similar to that of the calcium caseinate "remade" cream, the average churning time being from 40 to 45 minutes.

(d) *Tissue fibrinogen cream.* Tissue fibrinogen—a substance known to

[4] The calcium caseinate used in this experiment was an experimental commercial product manufactured as follows: Casein, precipitated by HCl and washed twice with water, was made up to 7–8 per cent total solids with water. At this time $Ca(OH)_2$ was added until litmus paper turned blue; the calcium caseinate suspension was heated to 160° F. and homogenized at 3000 lbs. pressure. The homogenized product was dried by a spray process, giving a very light, fluffy powder, easily dispersed in water to give a milky sol.

be a lecithoprotein (19)—was employed as emulsifying agent in several experiments in order to determine whether phospholipide-protein complex is important in the curd tension reduction of buttermilk. The experiments presented some unexpected problems regarding the stability and churnability of emulsions prepared by this agent, which first required solution. The starting material[5] was a concentrated paste and represented the globulin fraction which had been precipitated from a lung tissue extract by half saturation with $(NH_4)_2SO_4$. For making synthetic cream, a one per cent aqueous fibrinogen sol was employed. Since the fibrinogen paste contained a considerable amount of $(NH_4)_2SO_4$ the fibrinogen sol, prior to its use, was dialyzed for three hours against distilled water. At the end of this period the dialysate gave only a faint test for SO_4^{--}. Dialysis for a longer period caused the precipitation of globulin. The pH of the fibrinogen sol was 5.82. A preliminary experiment on the emulsification of butter fat in a fibrinogen sol at the pH of milk (6.64) showed that it was impossible to produce a normal cream emlusion. In view of this unexpected finding a study of the effect of the pH of the sol on the character of the resulting emulsion was undertaken with the following results:

1. *Emulsion with fibrinogen sol, pH = 5.82, or lower.* An unstable emulsion resulted, characterized by a pronounced clustering of fat globules, rapid rise of the cream and oiling off.

2. *Emulsion with fibrinogen sol, pH = 6.94.* The emulsion was even more unstable than in trial 1 of this series: it exhibited the same characteristics but to a greater extent.

3. *Emulsion with fibrinogen sol, pH = 7.30.* A fairly stable emulsion was obtained. The dispersion of fat globules was normal except for a small amount of clustering. There was no oiling off of the cream on standing.

The fibrinogen cream from which "remade" milks were made was, therefore, obtained by emulsifying butter fat in fibrinogen sol the pH of which was increased to 7.32 by adding NaOH. The separation of the "remade" whole appeared to be normal. The churning of the cream from the separated "remade" whole milk was characterized by the absence of foam and a relatively short churning time. The cream churned in less than 30 minutes.

The curd tension of the "remade" buttermilks from calcium caseinate and gelatin creams was zero in all cases as can be seen from Table 5. In the case of the "remade" buttermilks from these creams, as well as from some of the dried whey creams (see Table 1), total absence of clotting resulted when rennet was used as coagulant. The "remade" buttermilk from gelatin cream (experiment II) stood for an hour at 35° C. after rennet had been added yet remained perfectly liquid without a sign of visible coagulation. In experiment II (gelatin cream), after the first milliliter of rennet had been

[5] Fibrinogen was supplied by the Wm. S. Merrel Company, Manufacturing Pharmacists, Cincinnati, Ohio.

TABLE 5

Curd tension, pH, surface tension and composition of "remade" milks from skim milk powder, calcium caseinate and gelatin cream

Product tested	Curd tension	pH	Surface tension at 20–21° C.	Total solids	Fat	Solids-not-fat	Casein
	gms.		*Dynes per cm.*	%	%	%	%
(a) Skim milk powder cream:							
Original skim	84	.....	.....	.....	.....	.....	.....
"Remade" whole	64	.....	.....	.....	3.70	.....	.....
"Remade" skim	74	6.42	.....	10.03	0.26	9.77	.....
"Remade" buttermilk	49	5.88	.....	10.45	0.30	10.15	.....
(b) Calcium caseinate cream:							
Original skim	82	.....	.....	9.52	0.02	9.50	.....
"Remade" whole	60	.....	.....	.....	3.50	.....	.....
"Remade" skim	69	6.60	.....	9.94	0.06	8.88	.....
"Remade" buttermilk	*0*	6.21	.....	9.08	0.62	8.46	.....
Original skim diluted 13% with 3% Ca-caseinate sol (control)	68	.....	.....	.....	.....	.....	.....
"Remade" skim diluted 13% with 3% Ca-caseinate sol	58	.....	.....	.....	.....	.....	.....
(c) Gelatin creams: Exp. I							
Original skim	78	.....	.....	.....	.....	.....	.....
"Remade" skim	59	6.63	.....	8.55	0.03	8.52	.....
"Remade" whole	56	.....	.....	.....	3.10	.....	.....
"Remade" buttermilk	*0*	6.09	.....	9.12	1.40	7.72	.....
Original skim diluted 13% with 0.5% gelatin sol (control)	56	.....	.....	.....	.....	.....	.....
Original skim diluted 13% with H_2O	58	.....	.....	.....	.....	.....	.....
Exp. II							
Original skim	84	.....	.....	.....	.....	.....	.....
"Remade" skim	55	6.64	45.5	8.40	0.04	8.30	.....
"Remade" whole	42	.....	42.7	.....	3.70	.....	.....
"Remade" buttermilk	*0*	6.09	33.6	8.72	1.40	7.32	.....
Exp. III							
Original skim	73	6.64	47.6	.....	.....	.....	.....
"Remade" skim	48	6.64	46.9	8.28	0.02	8.26	2.30
"Remade" whole	46	6.52	43.6	.....	3.60	.....	.....
"Remade" buttermilk	*0*	6.10	38.5	10.08	2.80	7.28	1.94
"Remade" skim diluted 15% with H_2O	30	.....	.....	7.05	0.017	7.02	1.95
(d) Tissue fibrinogen cream:							
Original skim	79	6.61	46.9	.....	.....	.....	.....
"Remade" skim	57	6.65	46.2	8.20	0.02	8.18	2.48
"Remade" whole	49	6.63	.....	.....	4.07	.....	.....
"Remade" buttermilk	15	6.76	42.7	6.45	0.95	5.50	1.54
"Remade" skim diluted 32% with H_2O[1]	17	.....	.....	5.58	0.013	5.56	1.69

[1] Diluted to the per cent of solids-not-fat of the "remade" buttermilk.

added to the "remade" buttermilk and it had stood for 10 minutes at 35° C., the addition of two more milliliters of rennet to the "remade" buttermilk produced a partial coagulation but no clotting, as there was no separation of whey on standing. The "remade" buttermilk from calcium caseinate cream failed to give a visible coagulation after 20 minutes standing at 35° C. even on the addition of 3 ml. of concentrated rennet extract per 100 ml. of buttermilk.

All other "remade" milks, on the addition of rennet in the amount used in the determination of curd tension value (1 ml. of concentrated rennet extract per 100 ml. of milk), clotted within 30 seconds, usually instantaneously.

It is evident from Table 5 that the phenomenon of non-coagulation with rennet is restricted only to the "remade" buttermilks, since the curd tension of the other "remade" milks, as well as the curd tension of natural (original) skim to which gelatin or calcium caseinate sol had been added directly, was normal except for the effect of dilution. Furthermore, the above phenomenon is permanent, at least within the life of the product, since the samples of the "remade" buttermilk, when tested after standing six, fourteen, twenty-four, and forty-eight hours, all failed to coagulate with rennet. It should be kept in mind that the fat globule adsorption "membrane" in the case of gelatin creams, and especially in experiment III in which isoelectric gelatin was used, must have been composed entirely of protein material.

In the case of "remade" milks from skim milk powder cream, a reduction in curd tension of the "remade" buttermilk is evident; however, it is by no means so profound as in the case of the "remade" buttermilk from dried whey, gelatin or calcium caseinate cream.

The data in Table 5 on "remade" milks from tissue fibrinogen cream, the cream whose fat globule adsorption "membrane" was derived exclusively from lecithoprotein-complex material, show conclusively that this adsorption "membrane" had no effect on the curd tension of buttermilk. A rather low curd tension of the "remade" buttermilk is explained entirely by its low content of solids-not-fat because, when the "remade" skim was diluted to contain the same percentage of solids-not-fat as in the "remade" buttermilk, it gave an equally low curd tension. The low percentage of solids-not-fat in the "remade" buttermilk most likely is due to the retention of some of the cream plasma solids in the bowl of the separator in the process of separation of the "remade" whole milk since the fibrinogen-butter fat emulsion did exhibit a greater tendency for producing clustering of butter fat globules than the other creams.

Another important fact brought out by the experiment with tissue fibrinogen cream is the significance and the relation of pH and surface tension to the phenomenon of low curd tension, or total noncoagulation of the "remade"

buttermilk. As can be seen from Tables 1[6] and 2, in all cases in which the "remade" buttermilk had a low curd tension or did not coagulate with rennet, the pH and the surface tension of such buttermilk are significantly lower than the pH of other "remade" milks from the same cream. On the average, the pH was lower by from 0.5 to 0.6 units and the surface tension lower by about 7 dynes per cm. Surface tension of the tissue-fibrinogen "remade" buttermilk was only 3.5 dynes lower than that of the "remade" skim milk and the pH actually slightly higher.

The effect of addition of $CaCl_2$ on the curd tension of "remade" buttermilks. It is well known (20) that the addition of $CaCl_2$ to milk increases the rate of rennet coagulation, produces harder curd and speeds up the syneresis of the clot. This effect of $CaCl_2$ is due both to the lowering of the pH and to the increase of Ca^{++} in milk. The presence in milk of an adequate concentration of calcium ions is essential for a normal clotting with rennet.

The "remade" buttermilks which did not clot with rennet, clotted on the addition of $CaCl_2$ prior to the addition of rennet or on the addition of $CaCl_2$ in a form of pepsin + $CaCl_2$ (Hill's coagulant) or rennet + $CaCl_2$. In such cases a normal coagulation and wheying of clot took place, and the curd tension of the buttermilk was restored, at least partially, if not completely, in some instances. The magnitude of these curd tension values, as compared to the curd tension values of the corresponding other "remade" milks, is shown in Table 6.

It is interesting to note from the data in Table 6 that it is necessary to add to the "remade" buttermilks a rather large amount of $CaCl_2$ in order to obtain a clot. One ml. of 5 per cent $CaCl_2 \cdot 2H_2O$, which is equivalent to 13.6 mgms. of calcium added per 100 ml. of buttermilk, failed to produce a clot.

Apparently the explanation of the non-coagulation of the "remade" buttermilk in experiment II, gelatin group, Table 5, seems to be lack of Ca^{++}, yet, theoretically, there should have been more Ca^{++} in this "remade" buttermilk than in the other "remade" milks in the experiment since its pH was much lower. The problem may also be considered in relation to the Ca^{++} content of the products in the calcium caseinate experiment in which all the "remade" milks must have been actually higher in total calcium than the original skim milk. The most widely quoted mean value for the soluble calcium of milk (21) is 32 per cent of the total calcium, which would be about 30 to 40 mgms. of calcium per 100 ml. of milk. Only a portion of the soluble calcium exists in milk as Ca^{++} due to the occurrence of calcium citrate which is soluble but only slightly ionizable (20). Natural milk can be diluted with water to the extent of 40 per cent and, with a rennet concentration such as used in the

[6] The surface tensions of "remade" milks from whey powder cream (C) were as follows: "Remade" skim—49.0 dynes per cm., "remade" whole—46.2 dynes per cm., and "remade" buttermilk—38.5 dynes per cm.

TABLE 6

Curd tension of "remade" buttermilk on the addition of $CaCl_2$ as compared to other "remade" milks

Product tested	Curd[1] tension	Remarks
	gms.	
Calcium caseinate cream:		
Coagulant: Pepsin + $CaCl_2$		
Original skim	121	
"Remade" skim	95	
"Remade" whole	80	
"Remade" buttermilk	89	
Gelatin cream:		
Coagulant: Pepsin + $CaCl_2$		
Exp. I		
"Remade" skim	70	
"Remade" buttermilk	65	
"Remade" whole	57	
Coagulant: Rennet		
Exp. II		
"Remade" skim	55	
"Remade" whole	42	
"Remade" buttermilk + 1 ml. of 5% $CaCl_2 \cdot 2H_2O$[2]	0	Only partial coagulation, resembling precipitation rather than clotting.
"Remade" buttermilk + 4 ml. of 5% $CaCl_2 \cdot 2H_2O$	41	
Exp. III		
"Remade" skim	48	
"Remade" skim diluted 15% (diluted to the % of S.N.F. of the "remade" buttermilk)	30	
"Remade" skim diluted 15% + 4 ml. of 5% $CaCl_2 \cdot 2H_2O$	62	
"Remade" buttermilk + 4 ml. of 5% $CaCl_2 \cdot 2H_2O$	45	
Dried whey cream:		
Coagulant: Rennet		
"Remade" skim	48	
"Remade" whole	41	
"Remade" buttermilk	1	
Coagulant: Pepsin + $CaCl_2$		
"Remade" skim	57	
"Remade" buttermilk	25	
"Remade" whole	46	

[1] For curd tension values of "remade" milks from Ca-caseinate and gelatin creams, when rennet was used as a sole coagulant, see also Table 5.

[2] In all cases the stated amount of $CaCl_2$ added to 100 ml. of milk.

determination of curd tension, will still give a satisfactory clot. Of course, it must be appreciated that a decrease of ionizable calcium is not directly proportional to the degree of dilution because the dissociation would be greater in a more dilute solution.

Any discussion on the amount of Ca^{++} necessary to insure a normal clot with rennet is purely speculative since we have as yet no satisfactory means for measuring Ca^{++} in milk. However, on taking into consideration the history of the "remade" milks, we are justified in assuming that the cause of

non-coagulation of the "remade" buttermilks is not a low concentration of Ca^{++}. Further evidence in support of this is seen in Table 7 which gives the

TABLE 7

Calcium, phosphorus and ash contents of papain whey from "remade" buttermilk and "remade" skim milk of gelatin cream experiment

Exp.	Product	Milligrams per 100 gms. of whey		Ash
		Ca	P	
				%
II	Whey from "remade" buttermilk	36.80	45.40	0.404
	Whey from "remade" skim	29.98	40.02	0.450

Ca and P analysis of the whey from the "remade" buttermilk (gelatin cream, experiment II) in comparison with the whey from the "remade" skim. Although neither rennet nor pepsin would clot this "remade" buttermilk, it was found that papain, when used at proper concentration, would clot it with a satisfactory separation of clear whey. The optimum proteolytic activity of papain, according to Willstätter (22), lies at the isoelectric point of a protein. Kometiani (23), who used papain for obtaining whey from a heated milk, points out that the proteolytic activity of papain at the pH of milk is not strong enough to affect the calcium and phosphorus content of whey significantly.

The procedure for clotting milk with papain was similar to Kometiani's and, briefly, was as follows:

To 100 ml. of milk, warmed to 60° C., 5 ml. of 2.5 per cent aqueous solution of papain powder were added and the milk allowed to stand at 60° C. for 20 minutes. The separated whey was filtered and the calcium and phosphorus determined in duplicate 25 ml. aliquots of the whey from each sample.

On the addition of papain the "remade" buttermilk clotted almost instantaneously and the "remade" skim clotted in about four minutes.

The calcium content of the whey from the "remade" buttermilk was actually higher than that of the whey from the "remade" skim milk; this is to be expected since the pH of the buttermilk was 6.09 as compared with 6.64 for the "remade" skim.

SUMMARY

Synthetic creams were made by emulsifying pure butter fat in the following aqueous sols: Dried whey sol, skim milk powder sol, calcium caseinate sol, gelatin sol, and tissue fibrinogen (lecithoprotein material) sol. The resulting synthetic creams were dispersed in normal milk plasma to obtain "remade" whole milks which were separated to give the corresponding "remade" skim milks and "remade" creams. The "remade" buttermilks were prepared by churning the "remade" creams.

The fat globule adsorption "membrane" from dried whey cream, when released in buttermilk by churning the "remade" cream, exerted a profound effect on the rennet clot of the buttermilk. This effect was characterized either by the total inhibition of clotting or by the formation of very soft and fragile curd with little or no syneresis.

A still more pronounced effect of the fat globule adsorption "membrane" on the rennet clot of "remade" buttermilk was found in the cases of gelatin cream and calcium caseinate cream. "Remade" buttermilk from either of these creams invariably had no curd tension whatever and did not show even a visible coagulation on the addition of rennet.

Although the addition of $CaCl_2$, in rather large concentration, to buttermilks from the above creams restored somewhat their curd tension values, it has been shown by calcium and phosphorus analysis of the papain clot whey from the buttermilk of gelatin cream that the ultimate cause of this phenomenon of low curd tension, or non-coagulation, is more deep seated than that of lack of Ca^{++}. This being the case the $CaCl_2$ cannot be regarded as restoring the clotting properties in these experiments simply because it supplies Ca^{++} to the system.

The fat globule adsorption "membrane" from skim milk powder cream had only a relatively slight curd tension reducing effect on its "remade" buttermilk as compared with the effect exerted by the adsorption "membrane" from dried whey cream.

The fat globule adsorption "membrane" derived from tissue fibrinogen (lecithoprotein complex material) had no effect on the curd tension of "remade" buttermilk.

The phenomenon of total non-coagulation with rennet, or abnormally low curd tension of "remade" buttermilks, was always accompanied by considerably lower pH and lower surface tension of the "remade" buttermilks as compared with the pH and surface tension of their respective whole and skim milks. The factors involved in the decreased pH and lowered surface tension and their relation to the loss of clotting ability have been the subject of extensive further study by us.[7]

It may be stated briefly at this time that the total destruction of the clot in the case of certain "remade" buttermilks was found to be due largely to the liberation of certain fat acids and their adsorption by the calcium caseinate, preventing the clotting of the milk by rennin. It was also found that curd tension is not seriously affected, if affected at all, by butter fat hydrolysis except when an unnatural emulsifying agent is substituted for the natural fat globule adsorption "membrane" of cow's milk. Furthermore, when lauric, myristic or palmitic acid is added directly to skim milk or allowed to

[7] Part of this further study was a post doctorate work and was done by the senior author in the Dairy Chemistry Laboratory of the Dairy Industry Division, University of California, Davis.

develop as the result of hydrolysis of esters of these fat acids by "natural" esterase in the milk, the normal clotting properties of the milk are inhibited. Other important aspects of these findings will be presented in detail in the papers to follow.

REFERENCES

(1) STORCH, V. On the structure of the "fat globules" in cow's milk. Analyst 22: 197–211. 1897.

(2) VÖLTZ, V. Untersuchungen über die Serumhüllen der Milchkügelchen. Arch. Physiol. (Pfluger's) 102: 373. 1904.

(3) PALMER, L. S., AND SAMUELSSON, E. The nature of the substances adsorbed on the surface of the fat globules in cow's milk. Proc. Soc. Exp. Biol. Med. 21: 537–539. 1924.

(4) HATTORI, K. Ueber die Hüllensubstanz der Milchfettkügelchen. J. Pharm. Soc. Japan No. 516: 123. 1925. Chem. Abstracts 19: 2380.

(5) PALMER, L. S., AND WIESE, H. F. Substances adsorbed on fat globules in cream and their relation to churning. II. The isolation and identification of adsorbed substances. J. DAIRY SC. 16: 211–57. 1933.

(6) SCHWARZ, G., AND FISCHER, O. Beitrag zur Kenntnis der Hüllensubstanz der Fettkügelchen der Milch. Milchw. Forsch. 18: 53–67. 1936.

(7) WIESE, H. F., AND PALMER, L. S. Substances adsorbed on the fat globules in cream and their relation to churning. III. Analysis of the adsorbed protein. J. DAIRY SC. 17: 29–32. 1934.

(8) RIMPILA, C. E., AND PALMER, L. S. Substances adsorbed on fat globules in cream and their relation to churning. IV. Factors influencing the composition of the adsorption "membrane." J. DAIRY SC. 18: 827–839. 1935.

(9) PALMER, L. S., AND TARASSUK, N. P. The effect of the adsorption "membrane" around the fat globules on the curd tension of cow's milk. J. DAIRY SC. 19: 323–335. 1936.

(10) MORRIS, M., AND RICHARDSON, G. A. The production and use of soft curd milk. J. Pediat. 3: 449–460. 1933.

(11) MOIR, G. M. The determination of milk proteins. Analyst 56: 147–149; 56: 228–235. 1931.

(12) CAVETT, J. W. An improved micro-Kjeldahl method. J. Lab. and Clin. Med. 17: 79–80. 1931.

(13) MORRIS, H. P., NELSON, J. W., AND PALMER, L. S. Quantitative determination of calcium, magnesium and phosphorus in feedstuffs and cattle excreta. Improved technic. Ind. Eng. Chem. An. Ed. 3: 164–167. 1931.

(14) TARASSUK, N. P., AND RICHARDSON, G. A. Studies in rennin coagulation.—Factors effecting the nature of the clot. Abstract of Papers, 29th Annual Meeting Am. Dairy Sci. Assoc. p. 75. 1934.

(15) WEISBERG, E. M., JOHNSON, A., AND MCCOLLUM, E. V. Laboratory studies on the chemistry of soft curd milk. J. DAIRY SC. 16: 225–247. 1933.

(16) ESPE, D. L., AND DYE, J. A. Effect of curd tension on the digestibility of milk. Am. J. Diseases Children 43: 62–69. 1932.

(17) DOAN, F. J., AND WELCH, R. C. Soft curd milk. Pa. Agr. Exp. Sta. Bull. 312. 1934.

(18) CHAMBERS, L. A. Soft curd character induced in milk by intense sonic vibration. J. DAIRY SC. 19: 29–47. 1936.

(19) GORTNER, R. A. Outlines of Biochemistry, p. 400. John Wiley and Sons. New York. 1929; 2nd Ed. p. 491, 1938.

(20) ASSOCIATES OF ROGERS. Fundamentals of Dairy Science. Reinhold Publish. Corp. New York. 1935.

(21) ALLEN, L. A. The mineral constituents and citric acid content of milk. J. Dairy Res. 3: 1–51. 1931.

(22) WILLSTÄTTER, K. Untersuchungen über Enzyme. Vol. 2: 1547, 1629. Julius Springer, Berlin. 1928.

(23) KOMETIANI, P. A. Veränderungen einiger Milchbestandteile durch Erhitzen. Milchw. Forsch. 12: 433–454. 1931.

INADEQUACY OF A WHOLE MILK RATION FOR DAIRY CALVES AS MANIFESTED IN CHANGES OF BLOOD COMPOSITION AND IN OTHER PHYSIOLOGICAL DISORDERS[1]

G. H. WISE,[2] W. E. PETERSEN AND T. W. GULLICKSON
Division of Dairy Husbandry, University of Minnesota, St. Paul, Minnesota

Early quests have revealed that natural milk of the cow, though adequate in the early stages of the life of the calf, yields disastrous results if fed as the sole ration for any great length of time after the normal suckling period.

Numerous experiments have been conducted yielding much information relative to the deficiencies of whole milk as the sole diet for mammals. Since the extensive literature relative to the subject has been adequately reviewed and presented by Herman (15), no attempt is made to repeat or to condense the available facts. In the light of the results reported it was deemed advisable to extend the study of the various physiological changes manifested in calves restricted to milk rations. Special emphasis was placed on the changes in composition of the blood in an attempt to supplement and enlarge the reported symptomatology.

EXPERIMENTAL PROCEDURE

Selection of animals. Twelve calves as described in Table 1 were used in this experiment. Their dams were normal individuals receiving adequate rations consisting of grain and hay. The only requisites in the selection of the calves were good health and normal appearance. They were placed on experiment shortly after birth and retained until either death ensued or severe symptoms of malnutrition deemed supplementation of the ration advisable. In the latter case the calves were transferred to another experiment which will be reported in a later paper.

Feeding and management. All calves, with the exception of E–236, had continuous access to colostrum during the first two or three days after birth, the time they remained with their dams. Subsequently they were fed fresh raw milk by pail twice daily.

The milk was produced by the College Dairy Herd which consisted of Ayrshires, Jerseys, Guernseys and Holsteins, the last named predominating. The milking cows received medium quality alfalfa hay, corn silage, and a grain mixture consisting of 250 pounds of yellow corn, 250 pounds of barley,

Received for publication February 1, 1939.

[1] Paper No. 1682 Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] The data in this paper are from a thesis presented by G. H. Wise in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota, Minnesota Agricultural Experiment Station.

TABLE 1

Calves restricted to a whole milk diet

No. of animal	Breed	Sex	Age started	Age removed	Cause for discontinuance	Disposition
			days	*days*		
E–230	Grade Holstein	Female	3	103	Lethargy	Transferred
E–231	Grade Holstein	Female	3	196	Death	———
E–232	Grade Holstein	Female	3	273	Paresis	Transferred
E–233	Grade Holstein	Female	3	324	Stiffness	Transferred
E–234	Grade Holstein	Female	3	289	Anemia	Transferred
E–235	Grade Holstein	Female	4	98	Death	———
E–236	Grade Holstein	Male	6	270	Death	———
E–237	Grade Holstein	Female	3	55	Death	———
E–238	Purebred Guernsey	Male	8	161	Anemia	Transferred
E–239	Purebred Ayrshire	Male	3	181	Anemia	Transferred
E–240	Purebred Jersey	Male	2	97	Death	———
E–241	Purebred Holstein	Female	6	83	Tetany	Transferred

250 pounds of oats, 100 pounds of bran, 100 pounds of linseed meal, 50 pounds of cottonseed meal and 15 pounds of common salt. During the winter, the cows were confined to the barn, and during the remainder of the year, they were released into pasture which supplied not more than half the nutrient needs of the cows. The quantity of milk fed was regulated by the appetite of the calf and its subsequent response with respect to digestive disturbances.

Water generally was restricted. Only four, E–231, E–232, E–233 and E–234 received water, which was offered twice daily during summer and early fall. It was hoped that by withholding water the desire for milk would be increased. The validity of this assumption is doubtful. Commercial iodized sodium chloride was provided *ad lib*, but relatively small amounts were consumed.

The calves were confined in individual board-walled box stalls that were protected from direct sunlight. The concrete floors were kept well covered with wood shavings. The calves were not muzzled and consequently had free access to wood boards, shavings and hair. Since previous experiments (12) revealed that consumption of the foregoing materials does not vitiate the harmful effects of prolonged milk feeding, the calves were not muzzled.

Determination of various physiological changes. The recorded observations included thereon: (1) general health, (2) growth, (3) blood analysis and (4) post mortem examinations.

1. *General health.* A detailed record of the general appearance and the reactions of each calf throughout the experiment was made.

2. *Growth.* This was estimated by two common physical measurements, body weight and height at withers. Early morning weighings, before feeding, were made the first, tenth, twentieth, twenty-ninth and thirtieth days of the thirty-day experimental period. Height at the withers, the criterion of skeletal development, was determined the last day of the period.

3. *Blood analysis.* Blood was analyzed for corpuscular-plasma volume, hemoglobin and sugars. The plasma was analyzed for calcium, inorganic phosphorus, magnesium and fat. These analyses were made weekly. The anticipated advantages of this frequency were as follows: (1) permits each animal to serve as its own control, (2) establishes the time of change in any constituent, (3) detects the sequence of changes, and (4) reveals the duration of the change.

a. *System of preparing blood and plasma for analysis.* Samples of blood were taken from the jugular vein about three hours after the morning feeding. The blood was received into a 15 ml. centrifuge tube containing 0.15 ml. of saturated sodium citrate solution. Since the dilution was constant for all samples, this factor was not considered in any of the analytical results.

Portions of the citrated blood were withdrawn for hematocrit, for hemoglobin and for sugar determinations. The remainder was centrifuged to separate the plasma from the corpuscles.

In order to withdraw the maximum amount of plasma without disturbing the compacted cells, the plasma was removed by siphoning. (See Fig. 1.)

b. *Analytical procedures for blood and plasma.* The corpuscular-plasma volume was measured by the Wintrobe method (33, 34). According to this investigator the type of anti-coagulant plays a role in the volume of the individual corpuscles and, consequently, is an important factor that should be considered in the measurement of the total volume of packed cells. Green and Macaskill (13) contended that some plasma must always remain entangled in the midst of the compacted corpuscles regardless of the kind of anti-coagulant used. According to their calculations about one-third of the "red precipitated mass," designated as *volume of red cells,* is plasma. In view of the controversial status the volume per cent of cells presented herein are uncorrected for shrinkage of corpuscles.

Hemoglobin was determined according to the method of Newcomer (24). The filtrates for the blood sugar analysis were prepared by the procedure of Somogyi (30). The analyses of the filtrate were made according to the methods reported by Shaffer and Somogyi (25). The method employed for fat determination was that devised by Allen (1). The Clark-Collip (7) modification of the Kramer-Tisdall method was used for the determination of calcium of the plasma. The supernatant liquid from the precipitated calcium was preserved for magnesium determinations. The procedure followed in the magnesium analysis was that of Denis (10) with several adopted modifications (9). The phosphate of the precipitated magnesium ammonium phosphate was determined colorimetrically by the procedure of Bodansky (3). Inorganic phosphorus of the plasma was calculated from an analysis of the trichloracetic acid filtrate for phosphorus according to the method outlined by Bodansky (3). The major obstacle encountered in this procedure was an occasional turbidity of the filtrate. This was observed most frequently in

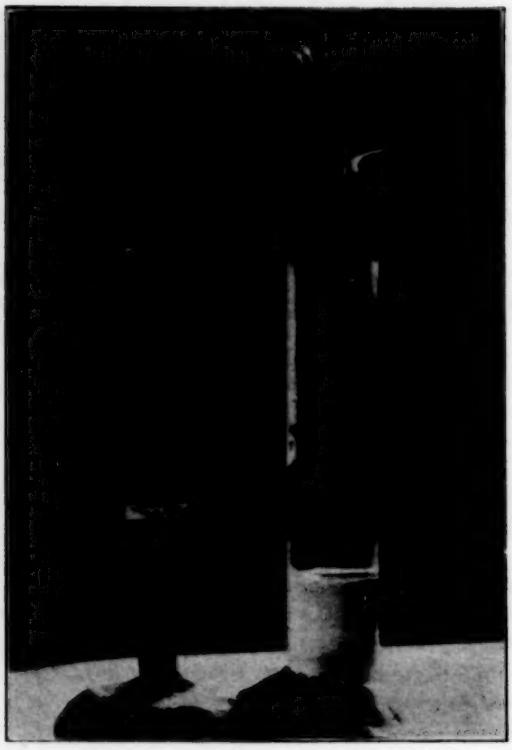

FIG. 1. Apparatus for removing plasma from centrifuged blood. The long leg of the siphon tube is extended through one of the openings of a three-hole rubber stopper. A short glass tube bent almost at right angles for connection to a suction pump and a straight short glass tube for vacuum control are inserted, respectively, into the remaining two openings. The stopper with its attachments is fitted tightly into the mouth of a test tube, the receptacle. By careful manipulation with this apparatus the plasma may be removed conveniently within 0.3 mm. of the cellular material without disturbance.

filtrates from plasma of very young calves. The difficulty was overcome somewhat by prolonged standing and refiltration.

4. *Autopsy.* Gross post mortem examinations were made within several hours after death of the experimental animal. Histological studies were made of selected organs, the liver, the kidneys, the spleen and the heart, from representative animals.

EXPERIMENTAL RESULTS

General health. All the calves apparently were healthy and vigorous during the first stages, from 25 to 60 days. Subsequently many symptoms

varying in kind and degree were manifested. In general, however, the sequence of changes are in accord with the reports of other investigators (15).

The gross symptomatology common to all the calves was first a depraved appetite as manifested in the insatiable desire to gnaw boards, chew shavings and lick hair. This abnormality was generally followed by the development of a long rough coat of hair, dry harsh skin, paleness of unpigmented areas, emaciation, sluggishness, occasional nervousness and anorexia. Colds and digestive disturbances were frequent and varied in duration and intensity with individual calves.

Some symptoms were restricted to a few individuals. Three of the calves, E–230, E–231 and E–232, experienced difficulty in swallowing. This symptom was very pronounced in the first two calves at 3 months of age but not in the latter until 9 months of age. There seemed to be a lack of coordination and synchronization in the deglutition process, frequently resulting in choking. This difficulty was alleviated somewhat by elevating the pail while the calf was attempting to drink. This symptom persisted until death in the case of E–231, which remained on the exclusive milk diet, and until the diet was changed in the cases of E–230 and E–232.

Other important gross symptoms encountered in various individuals were paresis, incoordinated locomotion as a consequence of lack of control of rear quarters, rachitic-like condition, edema of the joints, profuse lacrymation, excessive salivation, dyspnea, nasal discharges, nervousness, exophthalmus, tonic-clonic convulsions, retarded growth, chronic diarrhea and cachexia. The resistance of the calves to the syndrome varied considerably as suggested

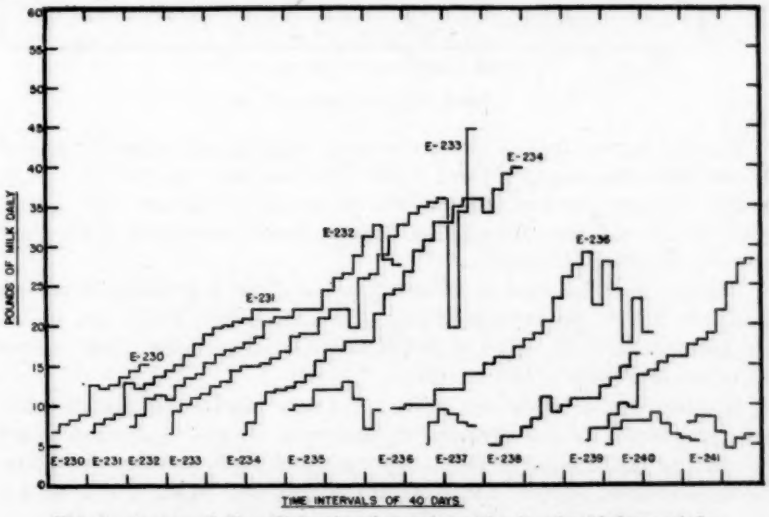

FIG. 2. Average daily milk intake of experimental calves by 10 day periods.

by the differences in the time they remained on experiment, shown in Table 1. The etiology in every case appeared to be of a dystrophic nature.

Milk consumption and growth of calves. The milk consumption, as presented graphically in Figure 2, was decidedly different for various calves. For the animals maintaining a good appetite the absolute daily intake was increased with advancing age, but the intake per unit of body weight actually decreased. Since the appetites of many of the calves were erratic, especially during the last stages of the experiment, the intake from day to day was variable.

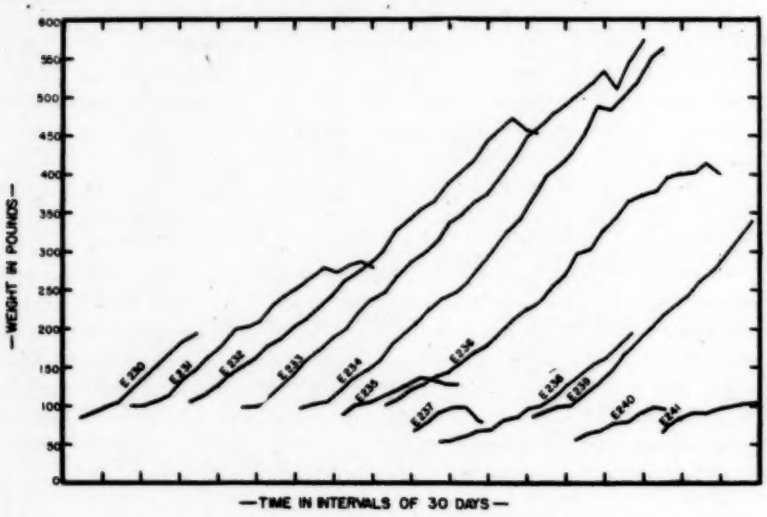

FIG. 3. Growth rate of experimental calves.

Growth, as measured by changes in body weight, is indicated in Figure 3. As with milk consumption, there is also much variation in rate of development. The extremes are probably due to two major factors, first, heterogenity of the experimental animals from a genetic standpoint and second, variation in nutrient intake.

Changes in composition of blood. Blood analyses were initiated too late to include E–230 and the early stages of E–231, E–232, E–233 and E–234. The composition of the blood of E–231 is presented in Table 2, and that of the others in Figures 4 to 8 inclusive.

Hematocrit and hemoglobin values. Anemia and low hematocrit values were pronounced in the advanced experimental stages. A supplementary examination of the blood of E–231 shortly before death revealed the following hematological picture: 2,900,000 erythrocytes and 10,440 leucocytes per ml., and a differential count of 84.5 per cent lymphocytes, 0.5 per cent mono-

TABLE 2

Composition of blood of E–231

Age	Blood			Plasma			
	Red blood cells	Hemoglobin	Sugar	Ca	Inorg. P	Mg	Fat
Days	*vol. %*	*gms. per 100 mls.*	*mgs. per 100 mls.*	*mgs. per 100 mls.*			
149	.....	.....	115.2	10.72	7.19	.....	.....
167	.....	4.92	127.0	9.94	4.69	.....	.....
179	.....	4.62	112.5	11.15	4.18	.....	129.3
186	.....	4.05	115.5	10.21	3.80	0.86	108.2
193	.....	3.23	120.0	9.99	3.26	0.93	109.3
194	14.17	3.01	167.0	12.01	3.52	0.72	121.9
196*	15.26	2.40	.....	12.39	3.40	0.97	47.3

* Died.

cytes and 15 per cent polymorphonuclear cells. Only two calves, E–235 and E–236 (Figs. 5 and 6), of seven calves studied within the first ten days after birth had hemoglobin values that could be considered within the normal range. The remaining five, E–237 to E–241, inclusive, had initial values between 4.43 gms. and 5.72 gms. The hemoglobin of calves having the initial high values gradually decreased to the level of the calves starting low; whereas, with only one exception, the hemoglobin of the calves having the initial low levels exhibited no significant variations. The exception, E–237, starting at the low level of 4.43 gms. attained a lower value of 2.09 gms., the lowest recorded in this investigation.

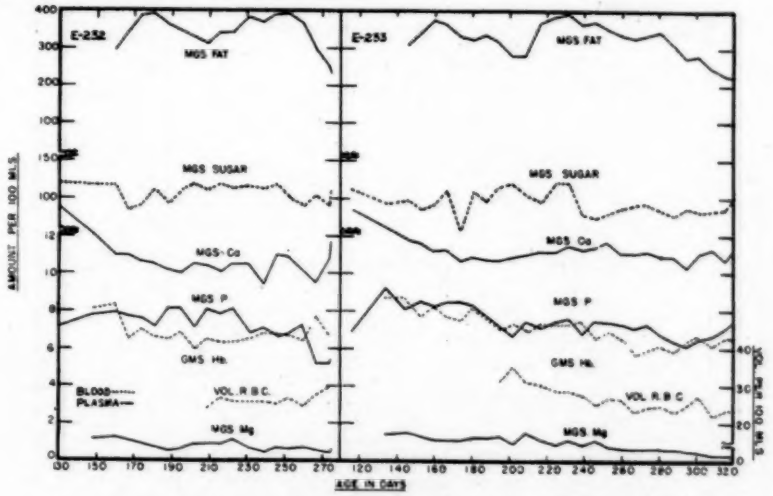

FIG. 4. Changes in composition of blood and plasma of E–232 and E–233.

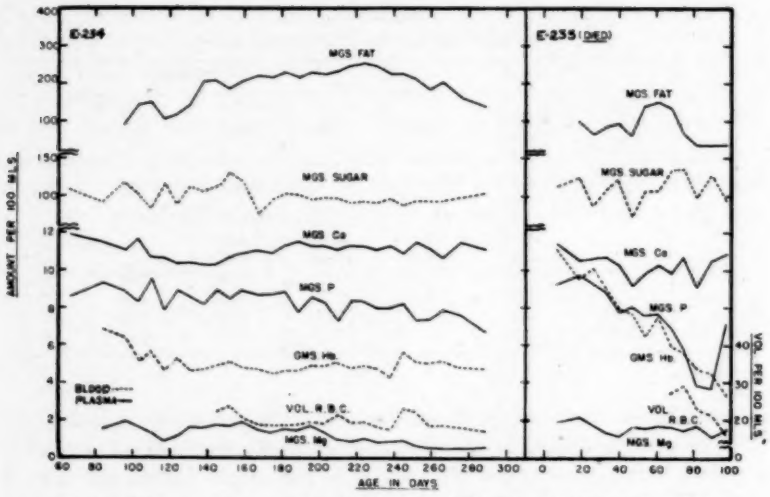

FIG. 5. Changes in composition of blood and plasma of E–234 and E–235.

Blood sugar. The data show that the levels for blood sugar of all calves were high, and that the values varied greatly with different individuals and in the same calf from time to time.

Fat of plasma. There was also a pronounced difference in the fat content of plasma in various calves. Generally the fat gradually increased to 200

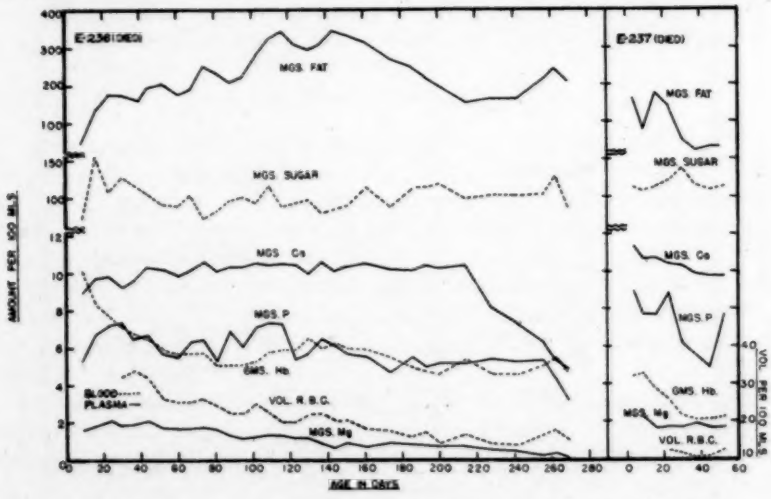

FIG. 6. Changes in composition of blood and plasma of E–236 and E–237.

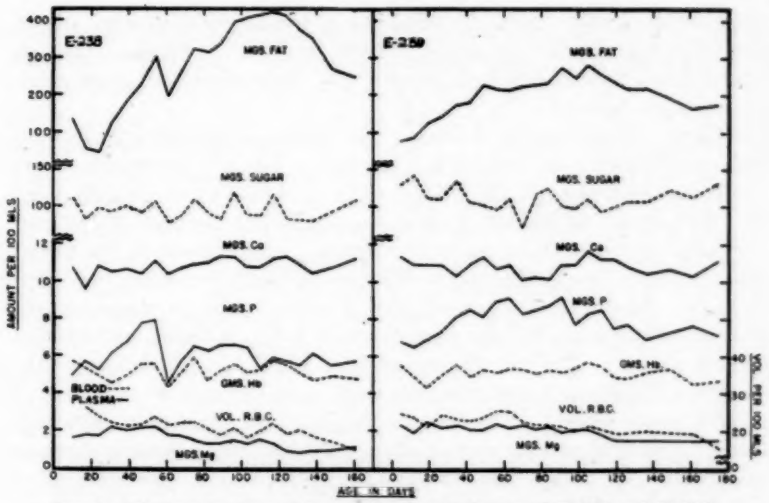

FIG. 7. Changes in composition of blood and plasma of E–238 and E–239.

mg. or more per 100 mls. if the calf remained thrifty. Otherwise the trend was up for a short time then downward and variable. During the early stages of life, the fat in accordance with the observations of Allen (2), was almost nil and practically devoid of pigments. The subsequent degree of pigmentation seemed to depend not only upon the quantity and quality of milk but also upon the individual calf.

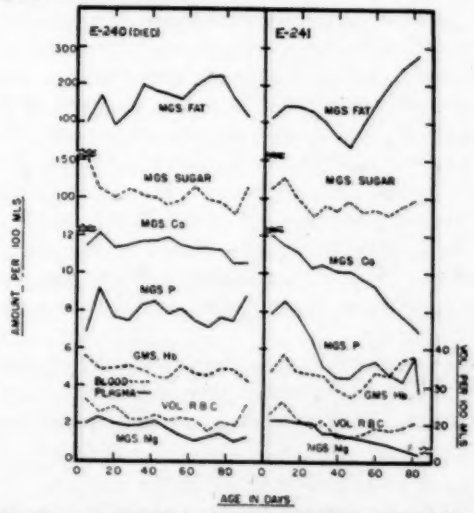

FIG. 8. Changes in composition of blood and plasma of E–240 and E–241.

Calcium of plasma. With the exception of two calves there were no evidences of abnormal calcium values. E–236 and E–241 attained the low values of 4.81 mgs. and 6.86 mgs. per 100 mls., respectively, at which levels both were convulsive.

Inorganic phosphorus of plasma. The inorganic phosphorus likewise varied considerably. As revealed in Figures 5, 6, 7 and 8, the extremes were 4.98 mgs. and 9.24 mgs. per 100 mls. for calves within the first ten days after birth. Subsequent trends were generally upward for a short period followed by a gradual decrease which rarely went below 6 mgs. However, the initial increase in two calves, E–235 and E–237, was followed by a rapid reduction until shortly before death, at which time a marked increase was observed. A gradual downward trend was observed in E–236 and E–241, terminating in tetanic seizures. The phosphorus levels of E–231, shortly before death, and those of E–232, in a semi-paralytic state, were also subnormal.

Magnesium of plasma. The important and perhaps significant fact is that within the first three or four months after birth there was a general decrease in the amount of plasma magnesium. Evidently in the cases of E–231, E–232, E–233 and E–234 a marked reduction of the element had taken place before blood analyses were applied. As may be noted, the rate and degree of reduction again emphasizes the varied responses of individual calves apparently subjected to similar treatment.

Post mortem examinations. Five calves ranging in ages from 55 to 270 days were autopsied. Examination of the digestive system revealed underdeveloped rumens in three cases and normal development in two others. Shavings, bits of curd, particles of loose hair, and hair balls were common ruminal contents. In every case there were lumps of undigested curd in the abomasum. In some cases the pyloric region was ulcerated. Generally the mucosa of the abomasum and the duodenum were slightly catarrhal.

Inspection of other organs revealed an atonic condition of the heart and petechial hemorrhages on the exterior surface of the auricular region in several cases. The spleens were generally atrophic, but the one from E–231 was decidedly hypertrophic. The adrenals in the cases of E–235 and E–236, were somewhat enlarged. Nephritis of calves E–235, E–237 and E–240, which died in an early stage, were characterized by numerous white spots on the kidney surface. With the exception of E–240 the livers were abnormal—anemic in appearance, hypertrophied and showed evidence of fatty infiltration.

The muscle tissue was generally pale and flaccid. The bones of the older animals, E–231 and E–236, both rachitic, showed evidence of osteoporosis on the articulating surfaces. Such symptoms were not evident on the bones of younger calves.

DISCUSSION OF RESULTS

The variability and complexity of the symptomatology render it difficult to assess the true significance of some of the results. The high and variable

blood sugar values reported herein are in accord with other results (16, 32) obtained from normal young calves. In agreement with the findings of Allen (2) working with normal animals, individuality and amount of fat consumed play important roles in the fat content of the plasma, but there are apparently additional factors involved. Digestive disturbances, for example, frequently cause marked reductions in plasma fat. Whether or not the metabolism of carbohydrates and fats as suggested by Sjollema (27) may be considered as a major factor in the etiology of the syndrome resulting from exclusive milk feeding remains a moot question. However, the results do not indicate that these two organic compounds play an important role.

The deficiency of iron and copper as manifested by anemia was very pronounced. Contrary to general findings, there were several cases of anemia in very young calves. These results are interesting in view of the belief that new-born animals are supplied with sufficient mineral reserves to tide them over the normal exclusive milk consuming period. However, in harmony with the reports of other investigators (15) normal hemoglobin levels are gradually reduced when the calves are restricted to milk rations. The complication of anemia with other symptoms render it difficult to establish a hemoglobin level below which death is likely to occur, but the data suggest that calves cannot tolerate values much below 3.00 gms. per 100 mls. of blood.

The interrelationship of calcium, phosphorus and magnesium and their relation to other constituents under the various conditions encountered render interpretation of results hazardous. The problems associated with the adequacy of these constituents in a milk diet and the degree of utilization by calves are still controversial.

This seems to be especially true of magnesium. The gradual reduction in the magnesium levels is in accordance with the reports from Michigan (11), Ohio (19) and Kansas (6) but contrary to the Missouri findings (15). A consideration of the theoretical needs of calves (11, 27) and the amount of magnesium in milk (20, 29) suggest that hypomagnesemia is the result of faulty utilization, the causes of which are unknown. Tufts and Greenberg (31) showed that diets high in calcium shorten the life span and increase the incidence and severity of the convulsive attacks of magnesium deficient rats. It is hardly conceivable that the Ca : Mg ratio in milk can be a major factor affecting the magnesium utilization by calves.

The hypocalcemia and the hypophosphoremia evidently were due to inefficient utilization of these elements. This might be attributed partly to an inadequacy of vitamin D (21) in the diet, especially during the winter season. Since diarrhea was frequently accompanied by a sudden drop in inorganic phosphorus it seems that digestive disturbances interfered with either absorption or retention of this element. In several cases there seemed to be a close relation between the trends of the phosphorus and the hemoglobin and the volume of erythrocytes. Though possible, the evidence is inadequate to suggest that an anemic condition may interfere with phosphorus metabolism.

It is difficult to ascribe any one of the multitude of gross symptoms specifically to the changes of any one constituent. Anemia and hypomagnesemia were common to all the calves but in different degrees. Though it has been demonstrated that tetany of magnesium deprivation is not due to an alteration in the calcium ion concentration of the blood (17), the tonic-clonic convulsions observed in E–236 and E–241 involved hypocalcemia and hypophosphoremia in addition to anemia and hypomagnesemia. Also the two cases showing rachitic symptoms were different as indicated by the calcium and the inorganic phosphorus of the plasma. For E–231 it was normal calcium—low phosphorus; whereas for E–236 it was low calcium—low phosphorus. The sudden increase of inorganic phosphorus following prolonged cachexia and shortly preceding the deaths of E–235, E–237 and E–240 may be attributed to initiation of protoplasmic disintegration liberating phosphorus into the plasma. These conditions are indicative of the complexity of the symptomology encountered.

It is likewise impossible to point to the specific factors responsible for the pathology of various internal organs. The so-called "spotted kidneys" observed in E–235, E–237 and E–240 may be attributed to either vitamin A deficiency (18) (22) (28) or to magnesium deficiency (8) (14) (23). The early appearance of the nephritic condition, the chronic digestive disturbances, the relatively high magnesium values of the plasma and the general unthrifty appearance are provisional evidence for listing vitamin A deficiency as the probable predisposing factor in the pathology of these calves.

Probably the cases of sudden change in the color of the plasma, from opalescence or very light yellow to a greenish brown, shortly before or during exhibitions of drastic physiological disturbances may be associated with the dysfunction of one or more organs. Brunner (5) reported that urobilemia produced a green fluorescence of the blood. Bollman and Schlotthauer (4) stated that an accumulation of bilirubin in the serum is observed when the liver or the bile ducts are infected. Though the evidence is very superficial, it is possible that liver and/or kidney disturbances were instrumental in the plasma pigmentation.

Another symptom that cannot be overlooked, is the general catarrhal enteritis and gastritis which obviously interfere with assimilation and subsequent metabolism. It remains to be determined whether the disturbances are due to the physical state of the milk in the digestive tract as suggested by Sheehy (26), to an inherent deficiency of the diet, or to an excess of some constituent in the milk.

SUMMARY AND CONCLUSIONS

When dairy calves, managed as outlined, are restricted for a prolonged period to a whole milk ration, the following abnormalities may be observed at various times:

1. General unthrifty appearance, anorexia, cachexia, dyspnea, occasional nervousness and tetany, rachitic condition, dysphagia, and digestive disturbances.

2. Marked variations in the growth rate.

3. Changes in the constituents of the blood involving high but variable values for blood sugar, extremes in fat of plasma but generally high values, decrease in hemoglobin, gradual downward trend of magnesium of plasma, occasional cases of hypophosphoremia, and rarely cases of hypocalcemia.

4. Abnormal condition of several internal organs—hypertrophied livers in which fatty degeneration is taking place, gastritis, enteritis, and nephritis.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the guidance and assistance of Doctors W. L. Boyd and F. H. H. Kernkamp, Division of Veterinary Medicine, in diagnostic work and in post mortem examinations.

REFERENCES

(1) ALLEN, N. N. A simple volumetric method for the determination of fat in blood plasma. Proc. Soc. Exper. Biol. and Med. 31: 991–993. 1934.

(2) ALLEN, N. N. Blood fat in dairy cattle. II. Blood fat as related to various physiological and environmental factors. Ph.D. thesis, University of Minnesota, St. Paul, Minn. 1935.

(3) BODANSKY, A. Determination of inorganic phosphate. Beer's law and interfering substances in the Kuttner-Lichtenstein method. J. Biol. Chem. 99: 197–206. 1933.

(4) BOLLMAN, J. L., AND SCHLOTTHAUER, C. F. Some blood chemical findings and their interpretations in diseases of animals. J. Am. Vet. M. Ass. 84(N.S. 37): 151–162. 1934.

(5) BRUNNER, W. The clinical significance of the green fluorescence of blood serum. Chem. Abs. 29: 5177. 1935.

(6) CAVE, H. W., RIDDELL, W. H., HUGHES, J. S., AND WHITNAH, C. H. Milk as a sole diet for calves. Eighth Bien. Rpt. Kan. Agr. Exper. Sta. p. 65. 1936.

(7) CLARK, E. P., AND COLLIP, J. B. A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem. 63: 461–464. 1925.

(8) CRAMER, W. Experimental production of kidney lesions by diet. Lancet (London) 2: 174–175. 1932.

(9) CUNNINGHAM, I. J., AND JOSLAND, S. W. The determination of magnesium in blood serum. New Zeal. J. Sc. and Techn. 16: 28–29. 1934.

(10) DENIS, W. The determination of magnesium in blood plasma and serum. J. Biol. Chem. 52: 411–415. 1922.

(11) DUNCAN, C. W., HUFFMAN, C. F., AND ROBINSON, C. D. Magnesium studies in calves. I. Tetany produced by a ration of milk or milk with various supplements. J. Biol. Chem. 108: 35–44. 1935.

(12) ECKLES, C. H., AND ASSOCIATES. Deficiencies of milk as an exclusive diet for calves. Unpublished data. Minn. Agr. Exper. Sta. 1920–1932.

(13) GREEN, H. H., AND MACASKILL, E. H. Studies in mineral metabolism. VI. Comparison of the blood of cow and calf in respect to mineral constituents. J. Agr. Sc. (England) 18: 384–390. 1928.

(14) GREENBERG, D. M., LUCIA, S. P., AND TUFTS, E. V. The effect of magnesium deprivation on renal function. Am. J. Physiol. 121: 424–430. 1938.

(15) HERMAN, H. A. Growth and development of dairy calves on a milk diet. Missouri Agr. Exper. Sta. Res. Bull. 245. 1936.

(16) HODGSON, R. E., RIDDELL, W. H., AND HUGHES, J. S. Factors influencing the blood-sugar level of dairy cattle. J. Agr. Res. (U. S.) 44: 357–365.

(17) HOOBLER, S. W., KRUSE, H. D., AND MCCOLLUM, E. V. Magnesium deficiency in animals. Am. J. Hyg. 25: 86–106. 1937.

(18) JONES, I. R. The role of vitamin A in the nutrition of calves. Ph.D. thesis, University of Minnesota, St. Paul, Minn. 1925.

(19) KNOOP, C. E., AND KRAUSS, W. E. Bone studies in dairy calves. Ohio Agr. Exper. Sta. Bull. 579, pp. 82 and 83. 1937.

(20) LAMPITT, L. H., BUSHILL, J. H., AND FILMER, D. F. Dialysis of milk. III. Salt equilibrium with special reference to calcium, magnesium and phosphorus. Biochem. J. 31: 1861. 1937.

(21) LONG, J. W., HUFFMAN, C. F., AND DUNCAN, C. W. A study of the vitamin D requirements of calves when natural milk furnished the sole source of the antirachitic factor. Milk Plant Monthly 25: No. 7, pp. 30–34 and 72. 1936.

(22) MOORE, L. A., AND HALLMAN, E. T. Production of white spotted kidneys in calves. J. DAIRY SC. 19: 434. 1936.

(23) MOORE, L. A., SHOLL, L. B., AND HALLMAN, E. T. Gross and microscopic pathology associated with low blood magnesium in dairy calves. J. DAIRY SC. 19: 441. 1936.

(24) NEWCOMER, H. S. Absorption spectra of acid hematin, oxyhemoglobin, and carbon monoxide hemoglobin. A new hemoglobinometer. J. Biol. Chem. 37: 465–496. 1919.

(25) SHAFFER, P. A., AND SOMOGYI, M. Copper-iodometric reagents for sugar determination. J. Biol. Chem. 100: 695–713. 1933.

(26) SHEEHY, E. J. Derangement of the digestive processes in the milk-fed calf due to abnormal curd formation in the fourth stomach. Roy. Dublin Soc. Sc. Proc. (N.S.) 21: 73–85. 1934.

(27) SJOLLEMA, B. Tetany in calves. Vet. J. 91: 132–137. 1935.

(28) SMITH, T. Focal interstitial nephritis in the calf following interference with the normal intake of colostrum. J. Exper. M. 41: 413–428.

(29) SOMMER, H. H., AND HART, E. B. The heat coagulation of milk. J. Biol. Chem. 40: 137–151. 1919.

(30) SOMOGYI, M. A method for the preparation of blood filtrates for the determination of sugar. J. Biol. Chem. 86: 655–663. 1930.

(31) TUFTS, E. V., AND GREENBERG, D. M. The biochemistry of magnesium deficiency. II. The minimum magnesium requirement for growth, gestation, and lactation, and the effect of the dietary calcium level thereon. J. Biol. Chem. 122: 715–726. 1938.

(32) VON TIECHMAN, H., UND LOGISCHEN. Studien über den Blutzuckergehalt als Leistungsmerkmal beim Rinde. Arch. f. Tierernähr. u. Tierzucht. 7: 532–570. 1932.

(33) WINTROBE, M. M. A simple and accurate hematocrit. J. Lab. Clin. M. 15: 287. 1929.

(34) WINTROBE, M. M. The size and hemoglobin content of the erythrocyte. Methods of determination and clinical application. J. Lab. Clin. M. 17: 899–912. 1932.

VALUE OF VARIOUS VITAMINS AND INORGANIC SALTS FOR OVERCOMING SYMPTOMS MANIFESTED IN CALVES RESTRICTED TO A WHOLE MILK RATION[1]

G. H. WISE,[2] T. W. GULLICKSON AND W. E. PETERSEN
Division of Dairy Husbandry, University of Minnesota, St. Paul, Minnesota

Since the discovery of the value of minerals and vitamins for growth and health, and even for life itself, many investigations have been conducted to ascertain the essentiality of these constituents in rations of cattle. Even with the vast amount of information acquired, the knowledge of the quantitative and qualitative needs of calves is still fragmentary. The dearth of specific information relative to the curative properties of various supplements when administered to calves manifesting dystrophic symptoms resulting from milk feeding provoked further investigation.

Previous results (23) revealed that in the syndrome developed from restricting calves to a whole milk ration anemia and hypomagnesemia were common. Less frequent were evidences of avitaminosis A and D. These symptoms in accordance with the results of other investigators as reviewed by Herman (9) suggested the advisability of adding vitamins, A and D, and the minerals, iron, copper and magnesium to the milk ration. Thus the primary object was to determine the responses of these malnourished calves to the addition of the foregoing constituents, individually or in combination.

EXPERIMENTAL PROCEDURE

The subjects, Table 1, were malnourished calves transferred from a previous experiment (23). The care and the management of these calves were virtually the same as already described (23). During spring, summer and early autumn two of the animals, E–233 and E–234, were released for exercise, water and sunlight into a small yard covered with gravelly earth and cinders but free of vegetative material.

The mineral elements were provided in the following salts: copper in cupric sulphate; iron in ferric chloride; magnesium in magnesium carbonate, magnesium chloride or magnesium sulphate; iodine in potassium iodide. A solution of cupric sulphate and ferric chloride was prepared so that the ratio of iron to copper was 10 : 1. The concentration was such that 1 ml. of solution contained 100 mgs. of iron and 10 mgs. of copper. Other solutions

Received for publication February 1, 1939.

[1] Paper No. 1680. Scientific Journal Series, Minnesota Agricultural Experiment Station.

[2] The data in this paper are from a thesis presented by G. H. Wise in partial fulfillment of the requirements for the degree of Doctor of Philosophy, University of Minnesota Agricultural Experiment Station.

TABLE 1

Description of calves; supplements fed; and duration of experimental treatment

No. of calf	Started on experiment		Supplements in order of addition	Removed from experiment	
	Age	Pronounced physiological state		Age	Cause
	days			*days*	
E-230	104	Lethargic; dysphagia	Magnesium sulphate	188	Death
E-232	274	Paresis; dysphagia	Magnesium chloride	331	Death
E-233	325	Hyperirritable; stiff	(1) C.L.O. + (2) $MgCO_3$ + (3) $FeCl_3$ & $CuSO_4$ soln.	720	Death
E-234	290	Anemic; hypomagnesemic	(1) $FeCl_3$ & $CuSO_4$ soln. + (2) $MgCO_3$ + (3) C.L.O.	555	Death
E-238	162	Anemic; exophthalmus	(1) $FeCl_3$ & $CuSO_4$ soln. + (2) $MgCO_3$ + C.L.O. + glucose	247	Sold
E-239	182	Anemic	(1) $FeCl_3$ & $CuSO_4$ soln. + (2) $MgCO_3$ + C.L.O.	235	Sold
E-241	83	Convulsive; emaciated; anemic	(1) C.L.O. + (2) $MgCO_3$ + (3) $FeCl_3$ & $CuSO_4$ soln.	234	Transferred

were 10 per cent magnesium sulphate and 50 per cent magnesium chloride, including water of crystallization in both cases. The low consumption of iodized salt prompted the inclusion of iodine which was prepared as a 1.3 per cent solution of potassium iodide. Since the solubility of magnesium carbonate in water is very low, the daily allotment of this salt for each calf was weighed in individual paper envelopes.

At the time this work was initiated, there were reports indicating that U.S.P. cod liver oil fed at moderate to high levels may be injurious to calves (6, 15, 21). In order to eliminate this possibly deleterious factor the concentrated oil, "Nopco XX," was fed.

Little information relative to the amounts of the foregoing supplements needed by cattle is available. The quantity of iron and copper provided was essentially the same as recommended by Knoop, Krauss and Washburn (13). When this investigation was started, the only suggestion of the quantitative needs of magnesium was the amounts supplied in normal rations (1). The standard arbitrarily adopted was to provide sufficient amounts of magnesium, usually magnesium carbonate, to maintain the level of the plasma magnesium between 2.0 and 2.5 mgs. per 100 mls. As a precautionary measure against possible iodine deficiency, 5 mls. of the 1.3 per cent potassium iodide solution were fed daily.

The manner of administration was usually orally, but in exceptional cases injection was employed in order to accelerate the reaction. The oral

administration was made by adding the supplements directly to the milk except when the animals had erratic appetites. In these cases the material was suspended or dissolved in water and given by drenching. The inorganic supplements were fed at morning feeding and the organic at evening.

The interval for drawing blood samples was extended from one week to two weeks.

EXPERIMENTAL RESULTS

The major consideration is the responses of the various calves to the supplements as manifested in general health and in the changes of blood composition, shown graphically in Figures 1 to 3, inclusive. As indicated in Table 1, there was little uniformity of animals transferred to this experiment. Likewise the subsequent reactions varied.

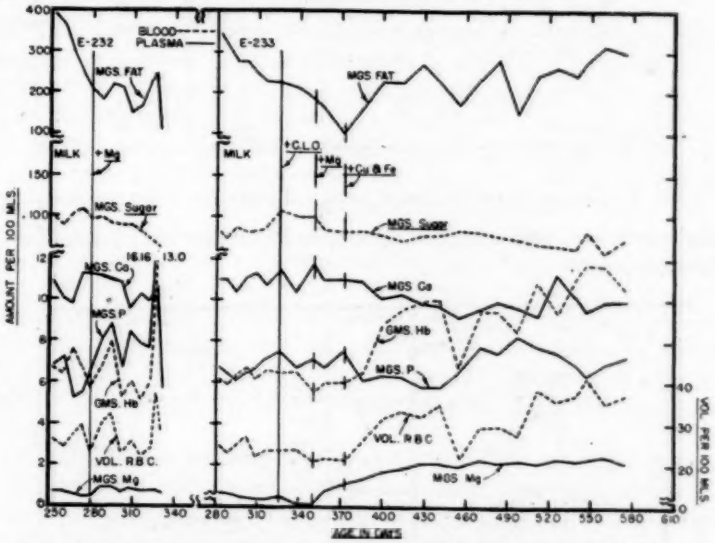

FIG. 1. Changes in composition of blood and plasma of E–232 and E–233.

Two calves, E–230 and E–232, received only magnesium supplements. The first, E–230, received 50 mls. of a 10 per cent solution of magnesium sulphate daily. The only observable changes were amelioration of the swallowing difficulty and increased vigor. Death ensued later following the sudden onset of a disturbance involving anorexia, constipation, abdominal distention and dyspnea. The increased concentration of the inorganic constituents of the blood, Table 2, and the development of a yellowish plasma pigment (23) shortly preceding death indicated marked physiological changes. Post mortem examination revealed a vast amount of fluid in the digestive tract but practically no liquid in the bladder. It appeared as

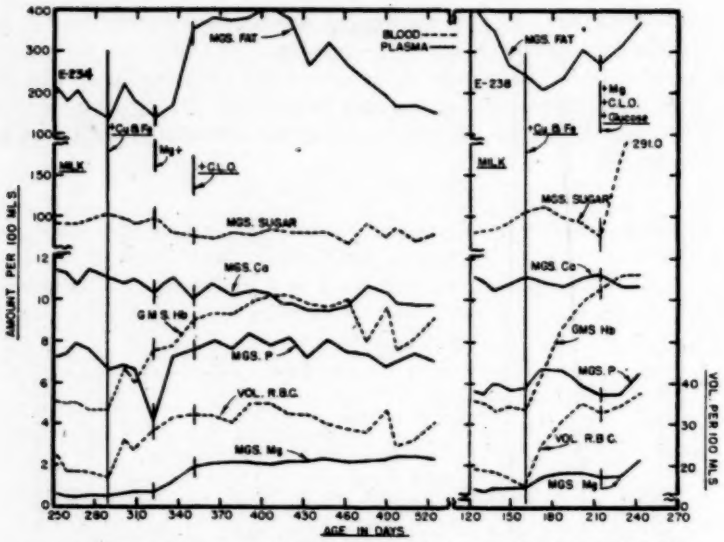

FIG. 2. Changes in composition of blood and plasma of E–234 and E–238.

though there had been a migration of fluids from the body tissues into the alimentary canal.

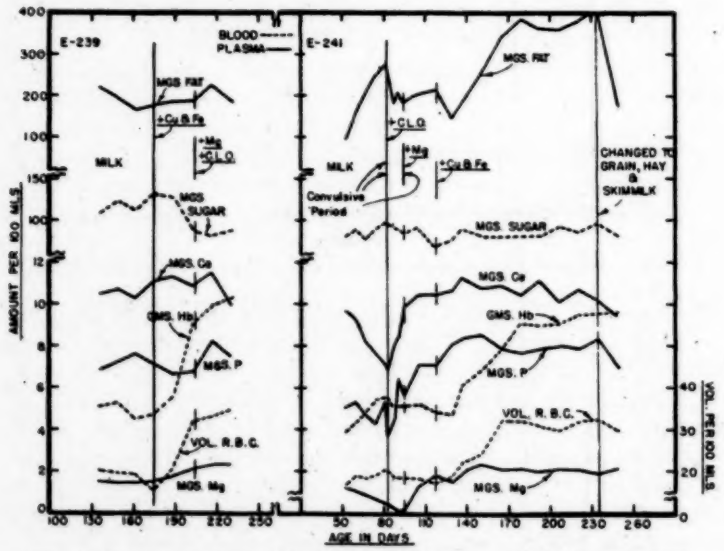

FIG. 3. Changes in composition of blood and plasma of E–239 and E–241.

TABLE 2

Composition of blood of E–230

Age	Blood		Plasma			
	Amount per 100 mls.		Amount per 100 mls.			
	Hemoglobin	Sugar	Ca	Inorg. P	Mg	Fat
days	*gms.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*	*mgs.*
154	.....	113.9	10.56	7.81	.....	.....
172	4.82	116.7	13.40	7.72	1.21	.....
184	5.41	111.5	11.74	8.08	0.97	313.5
188[1]	7.35	87.0	13.41	11.41	2.52	290.2
188[2]	.....	107.0	16.62	19.73	3.98	300.8

[1] Sample of blood drawn 2.5 hours before death.
[2] Sample of blood drawn 0.5 hour after death.

In the case of E–232 the syndrome included dysphagia, stiffness, enlarged joints and paresis accompanied by anemia and hypomagnesemia, Figure 1. In an attempt to relieve some of the symptoms, paresis particularly, several intravenous injections of a 10 per cent solution of magnesium chloride were made. This treatment aided in overcoming the semi-paralytic state. Subsequently magnesium chloride was administered orally. Dysphagia was relieved, but other disorders grew progressively worse. Digestive disturbances were frequent, especially following increases of the dosage of the supplement. The magnesium of the plasma was altered very little by the increased magnesium intake. In the final stages of life the animal acquired a dehydrated appearance, became very stiff, manifested alterations in blood including increased viscosity and pronounced changes in composition, Figure 1. Diarrhea and bloat, from gaseous putrefaction of a large amount of curd compacted in the rumen, apparently were the direct causes of death.

The supplements given to E–233, indicated in Table 1 and Figure 1, aided in relieving nervousness, stiffness and lassitude but did not overcome an erratic and depraved appetite. The elevation of the plasma magnesium level, following the addition of magnesium carbonate to the diet, and the increases of hemoglobin and red blood cells, following iron and copper supplementation were very marked. The sudden drop of the hemoglobin and the red cells on the 456th day was the result of a hemorrhage resulting from the removal of a large wart. Normal reproductive functions were evidenced by regular oestrus, by conception to second breeding, and by the presence of a well developed foetus (202 days of age) at the time of autopsy. The general health of the animal apparently was good until death at 720 days of age. The fact that a normally fed heifer in the same annex of the barn as E–233 died suddenly two days previously suggested that the cause of the death of E–233 was foreign to the experimental conditions. The post mortem examination revealed no abnormalities sufficient to cause death.

The responses of E–234 were similar to those of E–233. However, E–234 failed to conceive, but, as revealed in autopsy, her reproductive organs were normal. Though both animals possessed a depraved appetite, it was more marked in E–234. The excessive consumption of sand, gravel and cinders from the exercise lot produced unassessable damage that counteracted the recognized beneficial effects of sunshine and exercise. So much undigestible foreign material passed into the alimentary canal that rumenitis, acute gastritis and enteritis developed causing death at 18 months of age. Some nutritional deficiency might have been involved, but the mechanical irritation (1900 gms. of sand in the abomasum alone) was sufficient to initiate and aggravate the malady.

The remaining calves, two males and a free-martin, were removed from experiment as soon as they returned to normal. The two males, E–238 and E–239, responded quickly and profoundly to the supplementation. It is interesting to note the increase of the plasma magnesium following the addition of iron and copper. This response was also observed to a less extent in E–234. The increase may be attributed in part to the slight increase of magnesium intake resulting from a greater milk consumption. It is also conceivable that the supplements affected a more efficient utilization.

While E–241 was in the throes of a tonic-clonic convulsion, the blood composition was as shown in Figure 3. Calcium chloride was injected intravenously. This gave temporary relief probably as a consequence of a direct increase of calcium and an indirect increase of magnesium (19). Though calcium and inorganic phosphorus increased to normal following the addition of cod liver oil to the ration, convulsions persisted intermittently until magnesium was administered—first magnesium chloride intravenously followed by magnesium carbonate per os. This was the only direct positive evidence indicating that hypomagnesemia alone may be responsible for tetany (8). As in other cases, the response to copper and iron also was marked. Growth ceased during the period of the syndrome but resumed when the blood returned to normal composition.

Apparently neither the fat of the plasma nor the sugar of the blood was affected directly by the cod liver oil and the inorganic supplements. Though the fat frequently increased, this was the result of increased intake of milk and improvement of general health of the animal.

DISCUSSION

The addition of the minerals, copper, iron and magnesium, to the milk ration of calves manifesting symptoms of nutritional deficiency brought about profound changes in the health of the animals, in the hemoglobin of the blood and in the magnesium of the plasma. Additional supplementation with vitamins A and D, cod liver oil, no doubt contributed much to the improvement of health but induced no recognizable changes in the blood

other than the increase of calcium and phosphorus when these constituents were low. All the aforementioned supplements seemed to be essential for recovery and maintenance of health, particularly during the winter season.

The changes of the fat of the plasma and the sugar of the blood before (23) and following supplementation of the milk ration suggest that the metabolism of these two constituents are not directly involved in the syndrome. However, the level of the fat seems to vary with the intake of milk and the general health of the calves.

Of the many constituents considered in the etiology of the milk syndrome of calves, magnesium remains in the controversial limelight. The indispensability of this constituent in the diets of the animal organism is definitely established (19), but the amount needed by various species is unknown. This introduces the question of whether or not milk, when consumed to the extent of the appetite of a normal calf, supplies adequate magnesium for maintenance of normal functions. With one exception (9) the present evidence (3, 8, 11, 12, 23) indicates that calves consuming a milk ration that is not supplemented by magnesium become hypomagnesemic.

The etiological factor or factors involved in lowering the plasma magnesium constitute the crux of the problem. Neither Duncan and associates (8) nor Sjollema (20) attributed the lowered magnesium to an inherent deficiency of the milk but to a failure in the utilization of the available magnesium. Likewise Cunningham (5) has concluded, on the basis of the magnesium content of various feeds, that dietary deficiency is not the cause of the hypomagnesemia associated with grass staggers. Though the weight of evidence is against the postulation of an actual inadequacy of magnesium in milk, this factor cannot be dismissed until more is known of the absolute mineral needs of the calves. The intake of magnesium for calves consuming normal grain and hay rations (1) is about five times that of a similar calf receiving a whole milk ration. This obviously does not signify that the calf needs all the magnesium provided in the ration, but suggests the possibility of calves needing more magnesium than is now suspected. The high magnesium content (4, 12) of the bones of normal calves, and the stimulation of growth by milk also suggest the possibilities of increased needs of magnesium.

Perhaps the amount of magnesium normally eliminated is a factor that should be considered further in the adequacy of this constituent in the ration. Hirschfelder and Haury (10) stated, "normal animals and humans excrete about 42 per cent of ingested magnesium in the urine in 24 hours. The percentage of magnesium is fairly constant and is independent both of the dose and of the concentration of magnesium in the plasma." Boyd and Courtnay (2) observed that in the human subject all types of nephritis were accompanied by excessive loss of magnesium from the body. The

nephritic condition of calves (17, 23) associated with hypomagnesemia may be either the cause or the result of the decreased magnesium. Until further information is forthcoming, especially from metabolism trials, both the quantity of magnesium in the milk and the degree of utilization must be considered as etiological factors.

Regardless, the striking fact is that the addition of magnesium carbonate to whole milk rations of hypomagnesemic calves results in the restoration to the normal magnesium level. Huffman and Duncan (11) found that it was necessary to supplement milk with 15 to 20 mgs. of magnesium as magnesium oxide, per pound of body weight to maintain plasma magnesium values at normal levels. Only 8 to 10 mgs. were necessary when the source was natural feeds. In this investigation, after the calves recovered, a daily supplemental intake of five grams of powdered magnesium carbonate per 100 pounds of body weight (12.4 mgs. of Mg per lb. of weight) was adequate to maintain the plasma magnesium at a level not less than 2.00 mgs. per 100 mls. of plasma. The total daily intake per pound of body weight generally ranged from 13.0 to 18.0 mgs.

The only abnormality that was not overcome by incorporating the foregoing supplements in the milk was the depraved and erratic appetite. The adequacy of the supplemented milk ration for maintenance of other normal functions throughout a complete life cycle of the bovine remains unsolved. Unfortunately the results from the two subjects, E–233 and E–234, selected to test the permanency of the recovery from the deficiency syndrome were somewhat vitiated by the peculiar circumstances under which their deaths occurred.

Other minerals not included in the ration may be essential for sustenance of normal life functions. Since manganese, of which milk is known to be deficient, has not been demonstrated to be indispensible in the rations of cattle, this element was omitted. It is possible that this deficiency might have affected some important hitherto unrecognized metabolic functions. However, the essential rôle of manganese according to Von Oettingen (22) appears to be associated with reproductive cycles. According to Neal and Ahmann (18) cobalt is also an essential element in the rations of cattle. Calves receiving a basal ration of which dry skimmilk was a constituent manifested deficiency symptoms that were amenable to cobalt supplementation. This in conjunction with the fact that the presence of cobalt in milk has not been demonstrated (7) indicates that this mineral probably should be considered among the inadequate substances.

Other constituents including potent grass juice (14) and vitamin B_4 (9) have been proposed as supplements for raising calves on a liquid diet. The latter deficiency, vitamin B_4, was considered as a possible predisposing cause of chronic catarrhal gastritis and enteritis and ulceration of the pyloric mucosa. It is conceivable that some phase of the vitamin B-complex

may be involved. A study of the problem evokes several questions. Does the calf need the vitamin B-complex for normal physiological functions? If so, as applied to the problem of sustained milk feeding, does raw milk supply a sufficient quantity? Since Mangold and Usuelli (16) have demonstrated that milk rations cause the death of rumen infusoria, is it possible for the calves to synthesize the complex when they are restricted to whole milk rations? Until these questions are answered the vitamin B-complex cannot be disregarded as a factor in the problem under consideration.

SUMMARY AND CONCLUSIONS

1. Nearly all the recognizable symptoms of the syndrome developed in calves restricted to whole milk rations may be temporarily remedied by supplementing the diet with ferric chloride, cupric sulphate, magnesium carbonate and cod liver oil.

2. The adequacy of the supplemented ration for sustained normal functions throughout a life cycle including development, reproduction and lactation has not been demonstrated beyond the stage of advanced gestation.

3. The most pronounced abnormality that persisted after supplementation was a depraved and erratic appetite, which might have been the consequence of either a dietary deficiency or the physical nature of the ration.

4. The remedial results indicate that all the foregoing supplements are essential, but reveals little relative to fundamental etiology of the syndrome.

5. In view of the present evidence, it is probable that additional factors should be considered in the etiology of the pathological syndrome resulting from the inadequacy of milk rations for cattle.

REFERENCES

(1) BENNETT, F. B. The deficiencies of milk as an exclusive diet of calves. M.S. Thesis, University of Minnesota, St. Paul, Minn. 1932.

(2) BOYD, G. L., AND COURTNEY, A. M. Metabolism of salts in nephritis. II. Potassium, magnesium and sulfates. Amer. Dis. Child. 32: 29–39. 1926.

(3) CAVE, H. W., RIDDELL, W. H., HUGHES, J. S., AND WHITNAH, C. H. Milk as a sole diet for calves. Eighth Bien. Rpt. Kan. Agr. Exper. Sta. p. 65. 1936.

(4) CUNNINGHAM, I. J. The distribution of magnesium in the animal organism and the effect of dietary magnesium. New Zeal. J. Sc. and Techn. 18: 419–423. 1936.

(5) CUNNINGHAM, I. J. Grass staggers and magnesium metabolism. New Zeal. J. Sc. and Techn. 18: 424–428. 1936.

(6) DAVIS, G., AND MAYNARD, L. A. Cod liver oil and muscle dystrophy in calves. J. DAIRY SC. 19: 442. 1936.

(7) DINGLE, H., AND SHELDON, J. H. A spectrographic examination of the mineral content of human and other milk. Biochem. J. 32: 1078–1086. 1938.

(8) DUNCAN, C. W., HUFFMAN, C. F., AND ROBINSON, C. D. Magnesium studies in calves. I. Tetany produced by a ration of milk or milk with various supplements. J. Biol. Chem. 108: 35–44. 1935.

(9) HERMAN, H. A. Growth and development of dairy calves on a milk diet. Mo. Agr. Exper. Sta. Res. Bull. 245: 1–102. 1936.

(10) HIRSCHFELDER, A. D., AND HAURY, V. G. Clinical manifestations of high and low plasma magnesium and magnesium excretion after ingestion of magnesium sulfate. J. Biol. Chem. 104: 647–653. 1934.

(11) HUFFMAN, C. F., AND DUNCAN, C. W. Magnesium carbonate and magnesium oxide supplements to a whole milk ration for dairy calves. J. DAIRY SC. 19: 441. 1936.

(12) KNOOP, C. E., AND KRAUSS, W. E. Bone studies in dairy calves. Ohio Agr. Exper. Sta. Bull. 579, pp. 82 and 83. 1937.

(13) KNOOP, C. E., KRAUSS, W. E., AND WASHBURN, R. L. The development of nutritional anemia in dairy calves. J. DAIRY SC. 18: 337–347. 1935.

(14) KOHLER, G. O., ELVEHJEM, C. A., AND HART, E. B. The relation of the grass juice factor to guinea pig nutrition. J. Nutrit. 15: 445–459. 1938.

(15) MADSEN, L. L., MCCAY, C. M., MAYNARD, L. A., DAVIS, G. K., AND WOODWARD, J. C. Synthetic diets for herbivora, with special reference to the toxicity of cod liver oil. N. Y. (Cornell) Agr. Exper. Sta. Mem. 178, pp. 53. 1935.

(16) MANGOLD, E., UND USUELLI, F. Die Schödliche Wirkung der Milch und der veränderungen der H-Ion en Konzentration auf die Infusorien des Wiederküuermagens. Wiss. Arch. Landw. Abt. B. Tierernähr. Tierzucht. 3: 189–201. 1930.

(17) MOORE, L. A., SHOLL, L. B., AND HALLMAN, E. T. Gross and microscopic pathology associated with low blood magnesium in dairy calves. J. DAIRY SC. 19: 441. 1936.

(18) NEAL, W. M., AND AHMANN, C. F. The essentiality of cobalt in bovine nutrition. J. DAIRY SC. 20: 741–753. 1937.

(19) SCHMIDT, C. L. A., AND GREENBERG, D. M. Occurrence, transport and regulation of calcium, magnesium and phosphorus in the animal organism. Physiol. Rev. 15: 297–434. 1935.

(20) SJOLLEMA, B. Tetany in calves. Vet. J. 91: 132–137. 1935.

(21) TURNER, W. A., MEIGS, E. B., AND CONVERSE, H. T. Toxic effect of cod liver oil in the ration of the rabbit and the calf. J. Biol. Chem. 114 (Proc. Amer. Soc. Biol. Chem. 30): 104–105. 1936.

(22) VON OETTINGEN, W. F. Distribution and pharmacology of manganese. Physiol. Rev. 15: 197. 1935.

(23) WISE, G. H., PETERSEN, W. E., AND GULLICKSON, T. W. Inadequacy of a whole milk ration for dairy calves as manifested in changes of blood composition and in other physiological disorders. In press. 1939.

THE INFLUENCE OF THREE METHODS OF CLEANING THE CREAM SEPARATOR AND CREAM STORAGE TEMPERATURE ON THE KEEPING QUALITY OF CREAM[1]

W. J. CAULFIELD AND W. H. MARTIN
Kansas Agricultural Experiment Station, Manhattan, Kansas

INTRODUCTION

There is ample evidence in the literature to show that the use of an unwashed separator will result in highly contaminated cream from a bacteriological viewpoint. Great emphasis has been placed upon the importance of properly cleaning and sterilizing the separator after each time used. Under practical farm conditions, however, such an ideal procedure is frequently difficult to attain.

On many cream producing farms the general practice is to wash the separator but once daily. Improving the sanitary condition of the separator on the average farm is perhaps not so much a problem of education as it is in finding a procedure which will reduce the time and labor involved in cleaning the separator. Such a method has been proposed by Rogers and Evans.[2] Their method consists in immersing the separator parts continuously in a solution of trisodium phosphate and sodium chromate when not in use. Before immersing the parts in the solution they are rinsed with clean water and again when removed just prior to assembling the separator. No other cleaning treatment is involved.

Rogers and Evans reported this procedure reduced bacterial contamination of cream to a practical minimum. No serious corrosion of the metal parts resulted from the treatment. The effect of such a cleaning procedure on the flavor or keeping quality of cream was not determined. It would seem that such should be done before the method is generally recommended.

The principal objective of the investigation herein reported was to compare the effects of three procedures of cleaning and sterilizing the separator parts on the flavor and keeping quality of cream when held at three different storage temperatures.

PROCEDURE

In each of a series of nine trials split batches (10 gallons each) of whole milk were separated through three different separators, each of which had been cleaned by a different procedure. The three methods of cleaning cream separators compared in this study were: (1) Conventional, (2) Rogers, and (3) Unwashed.

Received for publication January 30, 1939.

[1] Contribution No. 127, Department of Dairy Husbandry.

[2] Rogers, L. A., and Evans, F. R. Cleaning dairy equipment with trisodium phosphate. J. DAIRY SC. 19: 733–738. 1936.

The conventional method consisted in thoroughly washing and steam sterilizing the separator parts before use. In the case of the Rogers method, the separator parts were immersed continuously when not in use in a solution composed of 5.0 pounds of trisodium phosphate, 0.25 pounds of sodium chromate, and 100 pounds of water. Except for rinsing the parts with a stream of water from a hose before placing them in the solution and again before assembling the separator no further cleaning treatment was involved. The unwashed method consisted in separating five gallons of whole milk through a clean separator on the day prior to the beginning of each trial and then leaving the separator unwashed over night.

The parts of each separator were cleaned by the same procedure for three consecutive trials and then were alternated so that during the course of the nine trials each separator was subjected to all three methods of cleaning. In this way it was possible to observe any effects of the cleaning treatment on the different metals used in the construction of the separator. In one of the separators all parts were constructed of stainless steel, another was equipped with stainless steel discs, whereas the third machine was constructed of tinned iron or steel throughout.

Each series of three trials extended over a two weeks period, during which the parts of the separator being cleaned by the Rogers method were immersed continuously in the solution when not in use, the same cleaning solution being employed for the entire period. To observe any accumulation of organic matter in the solution over a period of two weeks and to note whether the continued additions of organic material to the solution would have any influence on the effectiveness of the treatment, ten gallons of whole milk were separated daily and the parts placed in the solution after rinsing with water but not washed. Cream samples for comparative purposes, however, were taken only at the beginning and after one and two weeks respectively.

Each separator was adjusted to deliver cream testing 30–35 per cent fat. Each of the three lots of cream obtained was subdivided into three portions, placed into clean sterilized two quart glass fruit jars and stored in incubators adjusted to operate at 60, 70, and 80° F. respectively.

The various lots of cream were held at the temperatures indicated until they became second grade. Samples were removed daily, numbered so as to conceal their identity, and graded independently by two judges. The definitions given in the Kansas State Dairy Law for cream grades were used as a basis for grading. First grade cream is defined as cream that is clean, smooth, free from undesirable odors, clean to the taste and sweet or only slightly sour. Second grade cream is described as cream that is too sour to grade as first, that contains undesirable flavors and odors in a moderate degree that is foamy, yeasty or slightly stale, or that is too old to pass as first grade.

After the samples had been graded the acidity calculated as lactic acid was determined by titrating a nine gram portion with 0.1 normal NaOH using phenolphthalein as an indicator.

Immediately following the determination of the acidity 10 ml. of commercial formaldehyde, previously neutralized to the end point of phenolphthalein, was added to the cream and the mixture again titrated to the end point of phenolphthalein with 0.05 normal NaOH. This titration, known as the formol titration, is expressed arbitrarily as the ml. of 0.05 normal NaOH required to neutralize the carboxyl groups freed as a result of union of formaldehyde with the amino groups of proteins decomposed by biochemical processes.

RESULTS

Grades. It required from 7–11 days for the cream stored at 60° F. to become second grade when a clean separator was used, 5–12 days when cleaned by the Rogers method and from 2–9 days when an unwashed separator was used (Table 1). From the standpoint of keeping quality of the

TABLE 1

Days required for cream to change from first to second grade as influenced by the cleanliness of the separator and storage temperature (nine trials)

Method of cleaning separator	Temperature of storage					
	60° F.		70° F.		80° F.	
	Range	Average	Range	Average	Range	Average
Conventional	7–11	9.3	4–9	5.8	2–4	3.3
Rogers	5–12	8.5	2–8	5.5	3–4	3.4
Unwashed	2–9	6.5	1–7	3.6	1–4	2.5

cream, cleaning the separator by the Rogers method proved to be practically as effective as washing and steam sterilizing the parts just before use and was decidedly superior to the use of an unwashed separator. The use of an unwashed separator as compared with a clean machine reduced the average time required for the cream to change grade nearly three days when stored at 60° F.

The influence of the cleanliness of the separator on the keeping quality of cream was most apparent at the lower storage temperatures. When stored at 80° F. all of the cream became second grade in a relatively short time, averaging 2.5–3.4 days, irrespective of the procedure employed in cleaning the separator.

Temperature of storage was found to exert a greater influence on the keeping quality of cream than did the method of cleaning of the separator. Cream obtained with an unwashed separator remained first grade an average of 6.5 days when stored at 60° F., whereas cream from a clean separator remained first grade for only 5.8 days when stored at 70° F., and for only 3.3 days when stored at 80° F.

TABLE 2

Frequency of occurrence of flavor criticisms of cream samples at the time they were graded as second grade as influenced by the method of cleaning the separator and storage temperature (nine samples in each case)

Flavor criticism	Temperature of storage								
	60° F.			70° F.			80° F.		
	Method of cleaning the separator								
	Conventional	Rogers	Unwashed	Conventional	Rogers	Unwashed	Conventional	Rogers	Unwashed
High acid	3	3	5	9	5	4	6	7	5
Bitter	3	5	8	2	1	3	1	1	0
Yeasty	0	0	0	1	3	2	3	3	3
Unclean	1	1	3	1	0	3	0	3	3
Old stale	3	3	0	1	1	0	1	1	1
Metallic	2	0	1	1	1	0	1	0	1
Musty	1	0	0	0	2	2	0	0	0
Putrid	0	1	0	0	0	0	0	0	0
Cheesy	1	0	0	0	0	0	0	0	0

Flavor criticisms. The specific off-flavors together with the frequency of their occurrence in each of the different lots of cream at the time it was officially graded as second grade are recorded in Table 2. Since more than one flavor criticism was frequently applied to the same sample of cream the number of flavor criticisms exceeds the number of cream samples involved (Table 2).

The types of off-flavors which developed in the cream obtained from the separators cleaned by the Rogers method were no more objectionable than those which developed in the cream produced by the clean separators. Cream samples from the unwashed separator were criticized as "unclean" and "bitter" more frequently than the cream obtained from the separators cleaned by the two other methods. Aside from these two flavor criticisms, however, no definite trend in off-flavor development was observed to be associated with the method of cleaning the separator.

Temperature was found to have a marked influence on the type of off-flavors which developed in the cream during storage. The samples stored at 60° F. developed bitter flavors more frequently than at any other storage temperature. Yeastiness, on the other hand, was observed only in samples stored at 70 and 80° F., and was most frequent at the higher temperature. Samples criticized as "high acid" were usually those stored at the higher temperatures. Musty flavor occurred most frequently in the samples stored at 70° F. The storage temperature, therefore, not only has a marked influence on the time required for off-flavors in cream to develop but it may also govern the specific type of off-flavor.

Acidity. During the first 4 to 5 days of storage at 60° F. the cream obtained from the dirty separators increased in acidity at a faster rate than that obtained from the machines cleaned by the other two methods (Fig. 1). The greatest difference was observed after a two-day storage period. After the fifth day of storage at 60° F. there were no significant differences in the acidities of any of the samples.

As the storage temperature was increased from 60° F. to 70 and 80° F. the rate and extent of acid development in all cream samples was greatly accelerated irrespective of the procedure followed in cleaning the separator. The importance of using both a clean separator and low storage temperature for the production of high quality cream is emphasized by these results.

Formol titrations. The temperature of storage was found to have a greater influence on the rate of proteolytic decomposition, as measured by the formol titration, than did the method of cleaning the separator (Fig. 1). At each of the different storage temperatures the formol titrations were very similar for each lot of cream. As the temperature of storage was increased, however, from 60 to 70 and 80° F. the rate of proteolytic decomposition was accelerated in all samples irrespective of the method employed in cleaning the separators (Fig. 1).

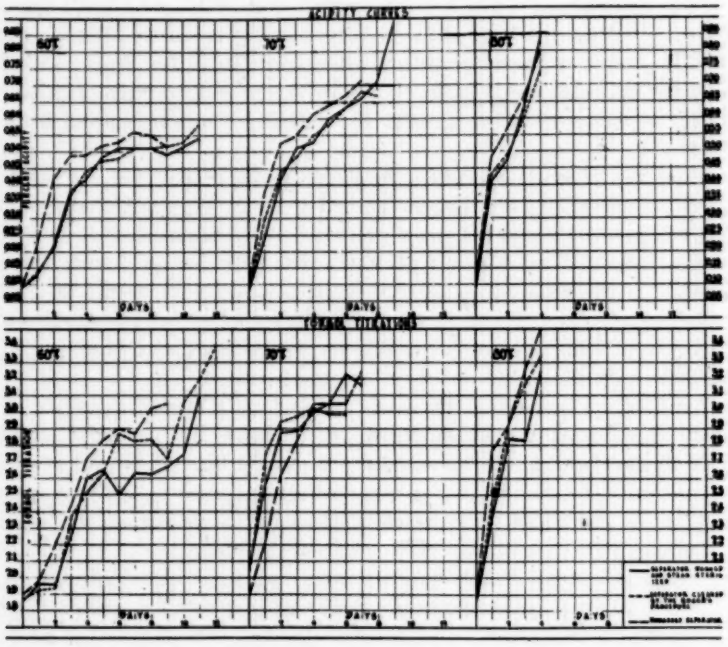

FIG. 1. Effect of three methods of cleaning the separator and temperature of storage on the titratable acidity and formol titration of cream

Effect on metals. Continuous immersion for two weeks in the trisodium phosphate, sodium chromate solution at a temperature of 85–92° F. had no visible effect on the separator parts constructed of stainless steel.

Similar treatment of the separator parts constructed of tinned steel showed some dark discoloration at the end of the two weeks period. The dark discoloration was particularly noticeable at soldered joints, although some of the discs showed discolored areas. The joints around the cream and skim milk spouts and feed cup were most seriously affected.

Strength and appearance of the solution. There was no appreciable depletion in the alkalinity of the trisodium phosphate, sodium chromate solution during a period of two weeks as measured by titrating a 5 ml. sample with 0.10 normal HCl solution using phenolphthalein as indicator. A reduction from 8.0 to 7.5 ml. of a 0.1 normal HCl solution was the greatest change in alkalinity observed in any of the trials. Such a slight change is of no practical significance as far as the cleaning and sterilizing properties of the solution are concerned. Aside from a slightly cloudy appearance at the end of the two weeks period no visible change was observed in the solution.

In no case was any objectionable odor observed nor was there any accumulation of organic material in the solution even though no attempt was made to remove completely the organic material from the separator parts before placing them in the solution.

SUMMARY AND CONCLUSIONS

Comparison has been made of three different procedures ((1) Conventional, (2) Rogers, and (3) Unwashed) of cleaning cream separators on the flavor and keeping quality of the resulting cream when stored at 60, 70, and 80° F. respectively. The data are based upon nine trials. The results are summarized as follows:

1. It required from 7–11 days for the cream stored at 60° F. to become second grade when a clean machine was used, 5–12 days when cleaned by the Rogers method, and from 2–9 days when an unwashed separator was used (Table 1).

2. The temperature of storage was found to exert a greater influence on the keeping quality of the cream than did the method of cleaning the separator. At 80° F. all of the cream samples changed from first to second grade in a relatively short time, averaging 2.5–3.4 days, irrespective of the procedure employed in cleaning the separator.

3. Samples of cream produced with the unwashed separator were criticised as having "unclean" and "bitter" flavors more frequently than those obtained from the other two separators. Cream obtained from the separators cleaned by the Rogers procedure did not develop off-flavors that were any more objectionable than those which developed in the cream produced by the clean separators.

4. Temperature of storage was found to have a significant influence upon the time required for off-flavors to develop in cream and also upon the specific type of off-flavor noted.

5. Temperature of storage was found to have a greater influence upon the rate of acid development and proteolytic decomposition in cream than did method of cleaning the separator.

6. Continuous immersion of stainless steel separator parts in the trisodium phosphate, sodium chromate solution for a period of two weeks at a temperature of 85–92° F. had no visible effect on the metal, but similar treatment of tinned steel or iron separator parts resulted in some dark discoloration particularly at soldered joints.

7. The daily additions of organic matter to the solution used in the Rogers method of cleaning over a two weeks period had no significant influence on its alkalinity nor on the color or condition of the solution.

VOLUME XXII JULY, 1939 NUMBER 7

JOURNAL OF DAIRY SCIENCE

Published by the

AMERICAN DAIRY SCIENCE ASSOCIATION

R. B. STOLTZ, Sec.-Treas.
Ohio State University, Columbus, Ohio

ABSTRACTS OF LITERATURE

T. S. SUTTON, Editor
Columbus, Ohio

MILK AND MILK PRODUCTS

Published in cooperation with

INTERNATIONAL ASSOCIATION OF ICE CREAM MANUFACTURERS
R. C. HIBBEN, 1105 Barr Bldg., Washington, D. C., Exec. Sec.

INTERNATIONAL ASSOCIATION OF MILK DEALERS
R. E. LITTLE, 309 W. Jackson Blvd., Chicago, Illinois, Exec. Sec.

Editorial Committee

H. H. SOMMER, Madison, Wisconsin, A. D. S. A.
J. C. HENING, Geneva, New York, A. D. S. A.
HAROLD PRATT, Philadelphia, Pennsylvania, I. A. I. C. M.
G. D. TURNBOW, Oakland, California, I. A. I. C. M.
W. D. DOTTERER, Chicago, Illinois, I. A. M. D.
A. J. POWERS, Brooklyn, New York, I. A. M. D.

CONTENTS

Bacteriology
Breeding
Butter
Cheese
Chemistry
Concentrated and dry milk; by-products
Diseases
Feeds and feeding
Food value of dairy products
Herd management
Ice cream
Milk
Miscellaneous
Physiology

THE SCIENCE PRESS PRINTING COMPANY LANCASTER, PA.

PUBLICATIONS AND ABSTRACTORS

EDITORS

Dahle, C. D., Fay, A. C., Holm, G. E., Marquardt, J. C., and Tracy, P. H.
Dahlberg, A. C.

ABSTRACTORS

Allen, N. N.
Archibald, J. G.
Atkeson, F. W.

Babcock, C. J.
Baltzer, A. C.
Barkman, J. O.
Bartlett, J. W.
Becker, R. B.
Bendixen, H. A.
Bennett, F. W.
Bird, E. W.
Burgwald, L. H.
Burri, R.
Brueckner, H. J.
Burke, A. D.
Bushnell, L. D.

Cannon, C. Y.
Carpenter, D. C.
Cave, H. W.
Clevenger, W. L.
Cole, W. C.
Copeland, L.
Coulter, S. T.
Cunningham, O. C.
Cunningham, W. S.

Dahlberg, A. C.
Darnell, A. L.
Demeter, K. J.
Doan, F. J.
Dorsey, L. M.
Downs, P. A.

England, C. W.
Erb, J. H.
Espe, D. L.

Fabian, F. W.
Frayer, J. M.
Frazier, W. C.
Fuller, J. M.

Geipl, A. J.
Golding, N. S.
Goss, E. F.
Greenbank, G. R.
Gullickson, T. W.
Guthrie, E. S.

Hansen, Arne
Hening, J. C.
Herrington, B. L.
Herser, F. H.
Holdaway, C. W.
Horrall, B. E.
Huffman, C. F.

Irwin, O. R.

Jacobson, C. O.
Jensen, Chris
Josephson, D. V.

Keith, J. I.
Knight, D.
Krauss, W. E.

LaMaster, J. P.
Leighton, A.
Lindquist, H. G.
Locke, R. B.
Lucas, P. S.
Lush, J. L.

Mack, M. J.
Macy, H.
Mann, A. I.
Marquardt, J. C.
Martin, W. H.
Mead, S. W.
Moore, L. A.
Morris, A. J.
Mueller, W. S.

Nelson, D. H.
Nelson, J. A.

Overman, O. R.

Palmer, C. C.
Parfitt, E. H.
Petersen, W. E.
Price, W. V.

Reid, W. H. E.
Richardson, G. A.
Riddell, W. H.

Schultz, E. N.
Sommer, H. H.
Stark, C. N.
Swope, W. D.

Tarassuk, N. P.
Theophilus, D. R.
Thomsen, L. C.
Thurston, L. M.
Totman, C. C.
Trout, G. M.
Tuckey, S. L.

Webb, B. H.
Weckel, K. G.
White, G. C.
Wilbur, J. W.
Wilster, G.
Wylie, C. E.

Yale, M. W.

JOURNALS

American Creamery and Poultry Produce Review
American Journal of Diseases of Children
American Journal of Physiology
American Journal of Public Health
Archives of Pediatrics

Biochemical Journal
Biochemische Zeitschrift

Canadian Dairy and Ice Cream Journal
Canadian Public Health Journal
Certified Milk
Cornell Veterinarian

Dairy Industries
Deutsche Molkerei Zeitung

Endocrinology

Food Industries
Food Manufacture
Food Research

Guernsey Breeders Journal
Ice and Refrigeration
Ice Cream Field
Ice Cream Industry
Ice Cream Review
Ice Cream Trade Journal
Industrial and Engineering Chemistry

Jersey Bulletin
Journal of Agricultural Research
Journal of Agricultural Science
Journal of American Chemical Society
Journal of American Veterinary Medicine Association
Journal of Bacteriology
Journal of Biological Chemistry
Journal of Dairy Research
Journal of Dairy Science
Journal of Experimental Medicine
Journal of General Physiology
Journal of Heredity
Journal of Genetics
Journal of Infectious Diseases
Journal of London Chemical Society
Journal of Milk Technology
Journal of Nutrition
Journal of Pathology and Bacteriology
Journal of Physical Chemistry
Journal of Physiology

Kaseindustrie
Kolloid-Zeitschrift

Lancet
Le Lait

Milchwirtschaftliche Forschungen
Milchwirtschaftliche Zeitung
Milk Dealer
Milk Industry
Milk Plant Monthly
Molkerei Zeitung

National Butter and Cheese Journal

Pacific Dairy Review
Proceedings of Society of Animal Production
Proceedings of Society of Experimental Biology and Medicine

Scientific Agriculture

Tierernährung
Tierzüchter
Trudy Vologodskogo Molochnogo Institut

Zeitschrift für Infektionskrankheiten Parasitäre Krankheiten und Hygiene der Haustiere
Zeitschrift für Physikalische Chemie, Abt. A and B
Zeitschrift für Untersuchung der Lebensmittel
Zeitschrift für Züchtung. Reihe B. Tierzuchtung und Zuchtungsbiologie
Zentralblatt für Bacteriologie
Züchtungskunde

SPECIAL PUBLICATIONS

Federal Dairying and Bacteriological Establishment, Liebefeld, Berne, Switzerland

International Association of Ice Cream Manufacturers
International Association of Milk Dealers

National Institute for Research in Dairying, Reading, England
New York Association of Dairy and Milk Inspectors

Prussian Dairy Research Institute, Kiel, Germany

State Agricultural Colleges and Experiment Stations

The Royal Technical College, Copenhagen, Denmark

United States Department of Agriculture

ABSTRACTS OF LITERATURE

BACTERIOLOGY

238. The Implantation of Oral and Intestinal Strains of L. Acidophilus in the Albino Rat. MICHEAL J. PELCZAR AND LUTHER A. BLACK, J. Bact. *37:* 1, 51–67, 1939.

Seven oral or dental strains of *L. acidophilus* were studied and none were found to be implantable in the intestine of white rats. Four of the five rough intestinal strains were found to be implantable. Rough intestinal strains were found to be more readily implanted and persisted in the intestine for a longer period of time than did the smooth intestinal strains. *L. bulgaricus* was found not implantable which is in accord with previous studies. E.H.P.

BUTTER

239. The Cooking-residue of Butter. W. RITTER, Schweiz. Milchzeitung No. 47, 1938.

The cooking residue of butter represents the condensed butter serum, which has been, so to speak, fried in the butterfat. It has a pleasant smell and taste and people like to eat it for instance spread on cake, or as an addition to fried potatoes. This cooking residue is composed of the lactose, the albumen and the salts of the butter. The relatively high content in butter phosphatides is particularly characteristic; the cooking residue of butter represents certainly the dairy product most rich in phosphatides that we know. It contains further 30–60 per cent of butterfat and varying amounts of water. On cooking sweet butter the cooking residue is quite sweet; acidified butter gives a residue with a pleasant sourish, and salted butter a residue with a salty taste. Lactose is present probably in the form of lactose anhydride. W.R.

240. Microscopical Examination of the Crystallization of Cooked Butter. W. RITTER. Schweiz. Milchzeitung No. 76, 1938.

Microscopical and polarisation microscopical examination of butterfat proved the following:

When it is cooled slowly, without stirring, or rapidly, in thick layers, butterfat forms centrally united bunches of needles, in which hundreds and thousands of crystal needles radiate from one point in all directions. These big, hedgehog like crystals are enclosed in an easily melting part of the butterfat, the butter-oil. They therefore do not form a solid mass, but are suspended more or less in the liquid basic substance. On the other hand

finely crystallized butterfat, as it is obtained by the inoculation method, consists in small single crystal needles which are entangled in every direction. Therefore the solid phase of the butterfat forms the basic substances in this case, while the liquid part, the butter-oil, is distributed between the little crystal needles. Hedgehog like crystals are always a symptom of bad crystallization, whereby insufficient solidity is prevailing, owing to the liquid basic substance. A slight warming (summer temperature) is enough to increase the amount of butter-oil, so that the coarse crystals fall to the bottom. If, on the contrary, the butterfat is cooled, the butter-oil concentrates; the upper parts of the hedgehog like crystals, spreading apart, consolidate and air is allowed to penetrate; thereby a white color is produced which is objected to by the consumer. On warming the fat the butter oil expands again, the air is removed and the normal color of the fat reappears. When the fat is heated more, the oil appears on its surface in the form of "tears." Such coarse, hedgehog like crystals have practically no inoculative effect if added to liquid fat cooled at 30–32° C. They may, however, be transformed into inoculative material by being carefully warmed at 36° C. under continuous stirring; thereby the accumulations of crystals separate in single crystal needles which may then initiate a good crystallization. W.R.

741. The Foaming of Fats, Especially Butter, When Used for Baking. W. Ritter. Mitteilungen Lebensmittel-Untersuchung & Hygiene *29*, 253–261. 1938.

Occasionally samples of butterfat are found which are foaming exceedingly when used for instance for the preparation of potato-chips. This foaming is due to an increased content in butter phosphatides. When butter or other phosphatides are added to oils or fats which do not foam, this phenomenon can be produced by heating the mixture with wet potatoes.

When cooked butter (ghee) is prepared, small amounts of butter phosphatides will pass, towards the end of the cooking process, from the cooking residue into the hot fat. They generally are separated again in a flaky or slimy form, when the butterfat is cooled at about 42° C. or when it is allowed to congeal and carefully warmed afterwards at 42° C. Then the phosphatides can be removed by filtration through paper. When the fat is heated anew, the phosphatides are dissolved again and run clear through the filter with the fat. There are sometimes samples of butter of a rather brown color, rich in phosphatides which they do not eliminate when cooled, etc. In such cases the phosphatides can be partially removed by a treatment with bone-black or other means by adsorption; this implies, however, that the butter pigment (carotene) is removed too. Better results are obtained by adding small amounts of water to the butterfat (less than 1 per cent). If well distributed in the butterfat, the water becomes absorbed by

the butter phosphatides which are thus "hydratised" and made insoluble in the butterfat. The phosphatides are removed by filtration in a swelled up state and the originally brown fat assumes a normal yellow color; at the same time there will be no more foaming, when the fat is heated with wet potatoes. The precipitation of the phosphatides which occurs quite normally when cooked butterfat is cooled, is due to its water content. The water being dissolved in the hot fat, a certain amount of phosphatides may be dissolved too. On cooling the hot fat, part of the dissolved water becomes insoluble, separates out and "hydratises" the dissolved phosphatides; these become insoluble too and are precipitated in a flaky or slimy form, according to their quantity and the water content of the butterfat. When butterfat is obtained by melting butter at about 42° C., it will be free from phosphatides, because the latter, if present in the fat, would have been immdiately "hydratised" by the water of the butter serum and made insoluble in the fat.

W.R.

242. Oxidation of Butterfat. I. The Formation of Peroxides in Butterfat. W. RITTER AND THS. NUSSBAUMER. Schweiz. Milchzeitung No. 12, 1938.

The beginning of the oxidation of butterfat can quite easily be fixed by determining the peroxide number. This latter, according to Lea, increases to a great degree when the fats are exposed to light. When air is made to pass through heated butterfat, the peroxide number increases more rapidly than without air. Copper, used as fat soluble copper palmitate, accelerates the assimilation of oxygen and increases accordingly the peroxide number of the fat, when this is stored in the dark at normal temperature. The higher the storing temperature, the quicker the increase of the peroxide number. The constancy of butterfat against oxidation is examined by heating 10 grams of the fat at 104° C. in a Petri dish during 8 hours and determining afterwards the peroxide number. Great differences will thereby result when butterfats of different constancy are examined. In general a butterfat is all the more constant against oxidation, the less the peroxide number increases in this test. Oxidizing fats distinctly grow in weight when heated. Therefore the determination of the percentage of water in butterfat must be carried out in the vacuum-drying stove. Fat extracted according to the Röse-Gottlieb method very often shows an extremely rapid increase of the peroxide number. If hydroquinon is added, the assimilation of oxygen in the light or while heating the butterfat is deferred for sometime. Butterfat obtained by melting butter at a low temperature gives a greater and quicker increase of the peroxide number than properly cooked butter. When tallowy oils or fats are intensely heated (for instance at 250° C.), the peroxide number decreases, probably because the peroxides of the fat are destroyed, that is to say, reacting with other groups of the fat. If,

on the other hand, normal butterfat is heated for a short time at 250° C., it will show afterwards at normal temperature or at 42° C. a quicker increase of the peroxide number than the same but not over heated fat. We may therefore conclude that overheating destroys certain elements contained in butterfat which are important for its keeping quality. W.R.

243. Oxidation of Butterfat. II. The Influence of Copper on the Peroxide number of the Butterfat. W. RITTER AND THS. NUSSBAUMER. Schweiz. Milchzeitung, No. 38, 1938.

It is demonstrated that an addition of increasing quantities of fat soluble copper palmitate to butterfat causes the peroxide number to increase all the more rapidly during the storage of the fat in the dark at ordinary room temperature, as the copper concentration is higher within the chosen range of 0.000001 to .33 per cent. If, on the other hand, the copper containing fat is stored at ordinary room temperature in the light, it shows a slower increase of the peroxide number than fat which is free of copper. Later on the peroxide number of copper containing butterfat decreases in spite of a continued exposure to light, which is not the case with copper free fat.

On heating the samples during 8 hours at 104° C., fats which contain about 0.0001 per cent of copper palmitate reveal the greatest and fat with a greater copper content a less important increase of the peroxide number. The reason for this phenomenon is that copper does not only accelerate the assimilation of oxygen on increasing the peroxide number, but also the further conversion of the fat peroxides. That is why the peroxide number decreases again in the presence of greater amounts of copper as well as in the light and in the warmth. Moreover, a distinctly tallowy butterfat, when heated at 104° C. during 8 hours, decreases all the more its high peroxide number, the higher the addition of copper palmitate. The nature of the second copper catalysis bringing about the destruction of the fat peroxides is not fully known. W.R.

244. Oxidation of Butterfat. III. The Influence of Different Substances on Peroxide-formation in the Butterfat. W. RITTER AND THS. NUSSBAUMER. Schweiz. Milchzeitung No. 82, 1938.

The influence of the peroxide number of different kinds of butterfat and the influence of additions to the butterfat were examined by heating 10 grams of the fat during 8 hours at 104° C. in a Petri dish of 10 cm. diameter. It resulted that butter shows a much slower increase of the peroxide number than pure butterfat. Whole butter very often has not oxidized after 24 hours to the same degree as pure fat after 8 hours. While the peroxide number of the pure butterfat rises herewith at about 40 on an average, that of the whole butter is only 1–3. This result is not due to evaporation of water, for it is scarcely affected by adding to pure butterfat an equal quan-

tity of water as contained in the whole butter. The butter serum must therefore contain elements which slacken the oxidation of butterfat. Properly cooked butter shows a considerably slower increase of the peroxide number than butterfat obtained by melting the butter at low temperatures. An ordinary cooking residue reduces the oxidation of the butterfat. A cooking residue extracted by benzol (removal of fat and butter-phosphatides) slackens but feebly the increase of the peroxide number. Lecithin and particularly cephalin obtained from plant lecithin or butter lecithin manifest a strong antioxidizing effect; dried egg yolk which is rich in lecithin does the same. "Avenex" added to butterfat in a dose of 1 per cent also produces an antioxidizing effect.

The antioxidizing effect of buttermilk and buttermilk-powder is relatively slight; on the other hand that of butter serum obtained by melting of butter is very strong. It follows that, on melting the butter, its antioxidizing elements are not transferred to the butterfat, but to the serum. Butter serum has a considerably higher phosphatide content than buttermilk and this is probably one of the reasons for the antioxidizing effect of butter serum. It follows equally from these statements that butter is more constant against oxidation than butterfat. Conditions in acidified butter are not yet cleared. W.R.

245. Oxidation of Butterfat. IV. Influence of Fat-peroxides on Keeping Quality of Butterfat. W. Ritter and Ths. Nussbaumer. Schweiz. Milchzeitung No. 92, 1938.

Oxidation may progress gradually from tallowy parts in the butterfat. This is due to the autocatalytic course of fat oxidation, which is accelerated by the developing fat peroxide. According to that the peroxide number increases slowly at first and more and more rapidly afterwards, until it has to slacken increase again. For this reason small amounts of already oxidized fat will reduce the keeping quality of normal butterfat. This was demonstrated by pursuing the course of the increase of the peroxide number in butterfat on adding 0, 1, 2, 5, and 10 per cent of tallowy butterfat at 20, 37, and 50° C. The influence of the oxidation accelerating effect of the fat peroxides became distinctly manifest, but it was not stronger than that of the storing temperature. The time needed for the peroxide number to reach a value of about 5–10 is very important for the determination of the keeping quality of butterfat. Later on, the natural protecting elements of the butterfat are no longer decisive for the assimilation of oxygen, the reaction taking an autocatalytic course. As it is difficult to fix the moment when the peroxide number reaches 5, the increase within a given time must be taken as a basis or else the time must be determined by means of the increase curve of the peroxide number. The turning white of the butterfat may serve as a hint that the peroxide number has approximately reached 5–10.

W.R.

246. The Congelation of Cooked Butter (Ghee). W. RITTER. Schweiz. Milchzeitung No. 93, 94, and 96, 1937.

In order to be solid and not to separate when exposed to warm summer temperatures butterfat must be as fine grained as possible. This is realized by cooling the hot butterfat at 30–32° C. and inoculating it with fine grained solid butterfat. After some time the mass, which is assiduously stirred, becomes thick and can be filled into the tins. If the butterfat is not properly cooled, coarse grains are formed which are suspended in a basic mass of liquid butter oil. It consequently occurs especially during warm summer temperatures that butter oil flows out of the tins and that a sabulous sediment is formed; this latter process is still hastened by a quick cooling at low temperature. When such a fat is warmed up, the butter oil sometimes appears at the surface of the mass in form of "tears." Butterfat that has not been duly cooled is liable to remain liquid at temperatures below 20° C., that is to say it can be poured from one tin into another and stirred as one likes. Properly cooled butterfat, on the contrary, is quite solid at those temperatures. The cooling of the fat, filling into tins and preparation of the inoculative material, etc., are described in detail. W.R.

247. About the Ways of Acting of Butter Made of Milk and of Butter Made of Separated Whey in Enzymic Reactions (Aldehyde-reductase- (Xanthine-oxidase-) reaction and Peroxidase-reaction). W. RITTER. Mitteilungen Lebensmitteluntersuchung und Hygiene *28*, 197–205, 1937.

Literature gives the following characteristics for butter made of milk and for butter made of separated whey in enzymic reactions: Milk-cream-butter (non-pasteurized) reduces immediately a mixture of formaldehyde and methylene blue, while butter made of separated whey and of whey the fat of which is collected by acidifying and heating (Vorbruchbutter) reduces very slowly or not at all. Milk-cream-butter and butter made of separated whey give a positive Storch reaction which takes place also, though more slowly, in butter made of whey the fat of which is collected by acidifying and heating. That butter made of separated whey which is not heated reduces but slowly a mixture of formaldehyde and methylene blue is therefore unexpected. On the other hand butter made of whey, the fat of which is collected by acidifying and heating, produces a positive Storch-reaction after the latter procedure.

According to the experiences of the author the copper-content of both kinds of butter is responsible for these anomalies, since they are in close contact with the copper kettle during the manufacturing process.

On examining the whey cream of cheese prepared in exactly the same whey, but in a copper kettle in one case and in a kettle of stainless steel in the other, it was stated that the above mentioned anomalies did not occur in

the latter case. The whey cream proceeding from the kettle of stainless steel showed a normal reduction time and no positive Storch reaction took place after the heating at curdling temperature. This cream did not develop tallowy flavor during cold storage, as was the case with the cream proceeding from the copper kettle. The aforesaid enzymic reactions applied for the differentiation of the kinds of butter are therefore only available for butter made in copper kettles, not for butter made in kettles of aluminium, tinned material or stainless steel. W.R.

248. The Importance of Pasteurization Temperature for Butter Making. W. RITTER. Alpenländische Molkerei- und Käserei-Zeitung *11:* 159–161, 1937.

On judging the effect of pasteurization on the quality of the butter, it is not only the bacterial result that must be taken into account. Other sides of the problem, the more chemical ones, are equally if not more important. So it was revealed by extensive experiments that butter made of cream pasteurized at 90° C., did not develop fishy flavor when stored, although the cream had been thoroughly acidified; if, however, the cream was heated at 78 to 82° C. only, the butter became fishy during the storing under otherwise equal conditions. This phenomenon cannot be caused by the destruction of the lecithin, the cooking residue of such a butter showing still a relatively high lecithin-content even after a long cooking at high temperature. It is also mentioned that analogous conditions arise on preparing other dairy products. W.R.

249. The Cooking of Butter. (Ghee-manufacturing.) W. RITTER. Reports of the World's Dairy Congress, Berlin, *2:* 156–161, 1937.

A short report is made on the ghee-manufacturing, the foundations and ways of cooking the butter, the phenomena occurring during the cooking-process, the keeping quality of butterfat and its ways of acting during the heating. Further on the differences between butterfat obtained by different methods, the crystallization of butterfat, the water content of cooked butter (ghee), the phosphatide content of differently obtained butterfats, bacterial defects, chemical defects of the product offered for sale, experiences made on controlling cooked butter (ghee). W.R.

250. The Testing of Starters in Dairy Plants. W. RITTER. Reports of the World's Dairy Congress, Berlin, Vol. *2,* 104–07, 1937.

Besides the examination of appearance, flavor and smell, the following methods of testing may be easily applied by dairy plants: Test on bacterial pureness according to Burri's smear culture method; test on development of carbonic acid by shaking the culture in a flask; determination of the degree of acidity, of the volatile acids and also of diacetyl by the Voges-

Proskauer method in the distillate of the determination of volatile acids, after their neutralization. The determination of diacetyl is carried out by steam distillation of 50 ccm. of the starter, 50 cc. of water and 3 cc. of N-sulphuric acid. Two samples of 10 cc. each of the distillate are titrated with n/20 solution of sodium hydroxide and phenolphthalein and tested on diacetyl by an addition of creatine and sodium hydroxide solution, eventually also α-naphthol solution.

A consumption of 0.60–0.85 cc. of N/20 sodium hydroxide solution obtained by neutralization of 10 ccm. of the distillate reveals a good starter, while lower figures indicate that the aroma streptococci have been restrained. When larger amounts of volatile acids are produced, acetone and diacetyl undergo in most cases further transformations, so that the reaction becomes more feeble or negative. W.R.

251. **The Means of Preventing Fishy Flavor.** W. RITTER. Reports of the World's Dairy Congress, Berlin, Vol. *2*, 162–167, 1937.

Fishy flavor occurs only in stored butter made of acidified cream. Therefore, acidification of the cream is generally omitted or the latter is neutralized before the churning, in order to prevent fishy flavor. The use of antioxidants, for instance hydroquinon, too would check this defect, but this is not legal. Certain bacteria are able to prevent oxidative changes in milk, cream, and butter. The most important method is, however, the application of an increased pasteurization temperature. Practical experiments carried out on a large base (6000 litres a day) in the central butter plant at Lucerne have revealed that fishy flavor developed when the cream was heated at 78, 80, or 82° C. in the flash pasteuriser; it did but rarely occur when the cream was heated at 86° C. and practically never appeared when the cream was pasteurized at 90° C. Similar observations were made in other butter plants on occasion of the taxations. These results are due to a chemical, not a bacterial effect of pasteurisation temperature. W.R.

252. **Holding Cream Raw and Pasteurized for Forty Hours.** C. C. TOTMAN, Am. Prod. Rev. 742–44.

Churnings of cream were neutralized and pasteurized and held 15 hours before churning; a second series were neutralized, pasteurized, and held 40–45 hours; while a third was held raw 40–45 hours, then neutralized, pasteurized, held two hours and churned. Cream held raw yielded higher scoring butter both when fresh and after six months' storage. This was especially true in the cases of lower grades and high acidity creams. With good cream the method of handling had little, if any effect. Several reasons for the results may be advanced: antagonistic action of lactic acid bacteria to proteolytic and lipolytic bacteria, and chemical action in the cream serum due to the effects of neutralizer, metallic salts, and heat. P.S.L.

253. How Fat Losses May Be Reduced. S. T. COULTER, Am. Prod. Rev. *85:* 25, 740–41, 1938.

Data are given from the operation of the Minnesota State Experimental Creamery. The author stresses three fundamentals: analysis of buttermilk from each churning and calculation of per cent butterfat lost of total fat received; training of employees in record accuracy and methods for reduction of fat losses; and, preparation of a chart to show weekly fat losses.

P.S.L.

254. A Skunk-Like Odor of Bacterial Origin in Butter. T. J. CLAYDON AND B. W. HAMMER, J. Bact. *37:* 3, 251–258, 1939.

From unsalted butter, which on holding at 21° C. developed a skunk-like odor, an organism, *Pseudomonas mephitica,* was isolated. This organism when inoculated in cream, pH 5.0–7.5, and churned, developed the defect in unsalted butter. Salt was found to have an inhibitory effect. The flavor could also be produced in unsalted butter by inoculating small quantities of a culture of *Ps. mephitica* into water used to wash butter. E.H.P.

255. An Anti-Oxidant for Dairy Products. C. D. DAHLE, Pennsylvania State College, State College, Pa., Nat. Butter and Cheese J., *29:* 24, 8, 1938.

Oxidized flavor is the most serious of all flavor defects of food. This flavor defect arises from the oxidation of fat. Oxidized flavor is associated with copper contamination of dairy products; exposure of such products to sunlight; the activity of an enzyme; and with the production of milk of low bacterial content. Cream, frozen for ice cream, is not susceptible to this defect. After all other precautions have been taken to prevent the occurrence of this flavor it may be still further inhibited by adding to the cream approximately 2 per cent of a specially prepared oat flour. Parchment paper, specially treated with oat flour, is recommended for preventing surface staleness in butter. Addition of oat flour directly to cream for buttermaking tends to prevent oxidized flavor. A water extract of the flour is recommended to avoid sediment in the butter. Oat flour also delays the formation of other flavor defects in butter, such as stale and cheesy flavors, which indicates that the oat flour may influence changes in constituents other than fat where an oxidation defect occurred. W.V.P.

256. Cooked Butter (Ghee). W. RITTER. Mitteilungen Lebensmitteluntersuchung und Hygiene, *28:* 206–14, 1937. (Conference held at the annual meeting of the Swiss Association of analytical chemists at Baden, June 11th, 1937.)

Pure butterfat is obtained by melting butter at about 40–50° C. and separating the fat from the aqueous parts, or by steam evaporation, which is the way of manufacturing ghee destined for sale in Switzerland.

Correctly cooked butter cannot develop bacterial defects, because the bacteria are lacking water, which is absolutely necessary for their propagation and vital activity. On the other hand cooked butter is more liable to oxidize than the original butter, because most of the elements which protect the fat against oxidation have gone into the cooking residue. However, the keeping quality of well cooked butter is by far better than that of butterfat melted at a low temperature. W.R.

CHEESE

257. Manufacture of Processed Swiss Cheese. C. R. BARKER, Nat. Butter and Cheese J., *29:* 24, 20, 1938.

Specific instructions for the selection of cheese for blending and processing are given. Desirable forms of cheese are described and certain precautions are mentioned to avoid certain defects. W.V.P.

DISEASE

258. Double-Zone Beta-Hemolytic Streptococci. J. HOWARD BROWN, J. Bact. *37:* 2, 133–144, 1939.

Double-zone beta-hemolytic streptococci which are found in the throats and vaginae of many normal persons are also the common cause of mastitis in cows. The organisms were found to fall into three fermentative groups and into Lancefield's serological group B. The recognition of the double-zone hemolytic streptococci is cited as a reason why diagnostic laboratories should not report all hemolytic streptococci as *Streptococcus hemolyticus.* E.H.P.

259. Die Uebertragung der Bruzellosen zwischen den Tierarten und dem Menschen. (Transfer of Brucella infection between animals and man.) ALEX THOMSEN. Zsch. Infektkr. Haustiere. *52:* 4, 281–315.

This paper is mostly a review of the literature on Brucella infection in man and animals. No definite conclusion or recommendations are given.

The geographical distribution of the three types of Brucella infection is very wide and a variety of animal species are susceptible to infection. This paper includes an extensive survey of the subject together with a discussion of prophylactic measures to control the infection in animals and man. L.D.B.

260. Differences in Immunization and Sensitization in Rabbits Injected with Relatively Avirulent or Highly Virulent Cultures of the same Strain (H) of Hemolytic Streptococcus. D. MURRAY ANGEVINE, J. Exp. M. *69:* 2, 211, 1939.

A number of reports have previously shown that virulent organisms are usually more efficient in the production of immunity than are avirulent organisms. This worker, in a previous report, had made the observation that an avirulent hemolytic streptococcus (strain B_1) when injected repeatedly into the skin of rabbits produced a high degree of sensitization associated with only moderate immunity, whereas rabbits injected with a virulent hemolytic streptococcus (strain H) were only slightly sensitive but had conspicuous immunity. Because the variations in the immune response may have been due to the strains used rather than to the virulence of the organisms, a further study was made using the same strain of streptococcus both in an avirulent and in a highly virulent phase.

These studies showed:

When a relatively avirulent strain of hemolytic streptococcus was injected into the skin of rabbits a considerable amount of skin sensitivity to streptococcus filtrate developed, and there was scant demonstrable immunity.

When the same strain of streptococcus was made highly virulent by repeated passages through rabbits, little sensitization and considerable immunity was produced. C.N.S.

261. Detection of the Virus of Poliomyelitis in the Nose and Throat and Gastro-Intestinal Tract of Human Beings and Monkeys. S. D KRAMER, B. HOSKWITH, AND L. H. GROSSMAN, J. Exp. Med. *69:* 1, 49.

The epidemiology of poliomyelitis strongly suggests the contact mode of infection. Experimental evidence furthermore points more specifically to the olfactory portion of the nasal mucous membrane as the portal of entry of the virus into the body.

Five strains of virus were recovered from nasal washings and feces. Four strains were of human origin, the fifth strain came from a monkey sacrificed at the height of the disease.

It is believed that the detection of the virus in the nasal washings of two additional patients during convalescence lends further support to the belief that the virus of poliomyelitis is spread by human contact.

The recovery of the virus from the feces 7 and 9 days after the onset of illness takes on added significance. It indicates first, that the virus withstands the gastric acidity which under normal physiological conditions tends to keep gastric contents relatively free of bacteria. It further suggests that improper disposal of feces from patients with poliomyelitis may have serious public health consequences, particularly in smaller communities where inadequate sewage disposal may result in contamination of surrounding beaches or even local water systems. C.N.S.

FOOD VALUE OF DAIRY PRODUCTS

262. Butterfat—Most Important Source of Vitamin A. JAMES MCLESTER. Am. Prod. Rev., *85:* 12, 308, 1938.

Four times the amount of Vitamin A required for absolute body needs is necessary for buoyant health. Butter yields 1,400 Sherman units per ounce. The daily minimum required for adults is 3,000 units more and is highly desirable. If butter alone furnishes the supply four tablespoons will provide the necessary amount. A pint of milk will supply 1,040 units, a dish of ice cream 670 units, and one ounce of cheese 700 units. The churning of butter does not cause a loss of Vitamin D. Storage of butter at low temperatures for as long as two years causes little, if any, loss of Vitamin A. P.S.L.

263. Dextrose in the Food Industries and Its Health Status. CARL R. FELLERS, Massachusetts State College, Amherst, Mass., Am. J. Pub. Health *29:* 2, 135, 1939.

The commercial manufacture of dextrose and its properties and uses in the food industries are described. Legal and public health aspects are discussed. Nutritionally, dextrose and sucrose are equivalent. From a sanitary point of view, essentially no problems exist relative to the use of dextrose. M.W.Y.

264. Bread and Milk—An Ideal Food. JAMES A. TOBEY, Am. Prod. Rev. *85:* 12, 313, 1938.

Some 35 to 40 chemical substances are necessary for adequate nourishment of the human body. Bakers, who add milk solids to bread, have performed a real service to nutrition, for such bread will supply two-thirds of the necessary materials. Bread, while energy-producing, makes possible more complete utilization of the protective food, milk solids. Neither bread nor milk are fattening foods. Milk solids in white bread causes it to approach more nearly whole wheat bread in nutritional value. P.S.L.

265. Effect of Milk Supplement on the Physical Status of Institutional Children. III. Progress of Dental Caries. L. J. ROBERTS, S. ENGLEBRECHT, R. BLAIR, W. WILLIAMS AND M. SCOTT. Am. J. Dis. Child. *56:* 805, 1938.

The effect of a milk supplement, with and without added Vitamin D, on the development of dental caries was observed for 90 children over a calendar year. The children were divided into three groups—one group remained as controls; the other two received daily per child a pint equivalent of evaporated milk, one having it in irradiated and the other in nonirradiated form.

All children showed extremely progressive caries. For the entire group there was a 26 per cent advance in number of carious teeth, a 50 per cent advance in number of cavities and a 98 per cent advance in the caries score.

The per cent age of increases in carious teeth was 28.7 for the control, 26.2 for the milk and 21.5 for the D milk group. The corresponding percentage of increase in number of cavities was 54.6, 46 and 50.7. These results indicate that the supplement tended to inhibit the rate of dental decay but that other factors also were involved. W.H.R.

HERD MANAGEMENT

266. Der Einfluss des Befruchtungszeitpunktes auf die Melk- und Trockenzeit. KALEFF, B. Zschr. f. Tierzücht. u. Zuchtungsbiol. *43* (1): 62–75, 1939.

Variations in service period (number of days from calving to the following conception) were studied in relation to milk production, to length of lactation after conception, and to length of the ensuing dry period, in 63 buffaloes, 146 cattle of the gray Bulgarian race, and 144 red cattle of mixed descent in the herd of the agricultural college at Sadowa. Some general observations on sheep are also included. Early rebreeding was most profitable. The weight of the newborn calves was not affected by the length of the preceding dry period nor by any circumstance other than extreme sickness of the dam. Cows kept for both work and milk conceived again sooner than those kept for milk alone. Some Bulgarian sheep produce lambs twice a year but others lamb only once. The difference is thought to be mainly in their heredity. Differences in regularity of calving among cows are also thought to be in considerable part hereditary and worth attention when selecting for breeding purposes. The general level of milk production in these herds was not high, the annual mean for the buffaloes being about 3,000 pounds. J.L.L.

ICE CREAM

267. The Institute Is Working for You. BERT SWEETING, Medosweet Dairies, Inc., Tacoma, Wash. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 7, Oct., 1938.

The activities of the Ice Cream Merchandising Institute are briefly reviewed. A series of merchandising short courses have been held in various cities in the country. A booklet entitled, "Let's sell Ice Cream" has been published. A testing laboratory has been set up in Washington for testing and perfecting new ways of serving ice cream at soda fountains. The two regular publications of the Institute, "Spinning Wheel" and "Ice Cream Currents," have been continued and improved. M.J.M.

268. The A B C's of Selling. NEAL BOWMAN, Temple University, Philadelphia, Pa., Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 35, 1938.

The following definition for selling is given: Salesmanship is selling goods that will not come back to people who will come back. About 39 per cent of all the profit in merchandising is said to be lost on returned merchandise.

The author then illustrates each letter in the alphabet with a word representing some phase of selling, which he develops in an interesting manner to emphasize the important points in the psychology of salesmanship.

M.J.M.

269. Don't Sell the Steak—Sell the Sizzle. ELMER WHEELER, Tested Selling Institute, New York, N. Y. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 23, 1938.

This is an interesting presentation of the subject of, "What Makes People Buy?" The author discusses various tested methods which have been applied successfully to retail merchandising. M.J.M.

270. The Ice Cream Industry's Opportunity in the Present Consumer Movement. MISS AUBYN CHINN, The Borden Co., Chicago, Ill. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs. *4:* 11, Oct., 1938.

The author lists the principal professional and consumer groups of women and gives the approximate membership of each group. The present consumer movement had its inception in the depression. Every consumer group with any leadership is definitely taking part in influencing legislation.

The consumer wants business to take an interest in her wishes for information and in her buying problems. For self protection the ice cream industry should take advantage of this desire. The consumer is interested in having this information about any product: 1. Facts behind the manufacture of the product, 2. Quality, 3. Price, 4. Appearance, and 5. Care of the product.

There is no industry that has more thrilling story to tell the consumer. The higher the quality the more zealous the ice cream manufacturer should be to encourage the consumer to inquire into the facts behind the product. There are all types of ice cream on the market today. Responsible manufacturers should encourage the discovery on the part of the consumer of those factors which make the difference between a good product at a fair price and a poor one. M.J.M

271. Organization Building. IRVING C. REYNOLDS, Franklin Creamery Co., Toledo, Ohio. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 46, 1938.

Business has a very definite responsibility, which is to create and conserve human values to match its other achievements. In so doing it is necessary to develop and inspire leadership of sound character.

Certain incentives which have been found to be helpful are:—

1. Leadership—to be put in positions where they lead seems to develop greatly some people.
2. Promotion—looking forward to the realization of deferred payments benefits some workers.
3. Regular pay—with some it is the first consideration but not to others.
4. Commission or piece work—many people like to feel that their efforts are being measured.
5. Bonuses or prizes—as a rule stimulate effort for a short period of time.
6. Profit-sharing—a principle given great publicity but not very important in actual operation.
7. Power over things or over people—a stimulus to many.
8. Research or study—many people have a craving to develop themselves in their industry.
9. The family spirit—the feeling of unity in an attempt to succeed invaluable.
10. Unionism—this means a great deal to many people and the principles of the strength of the union cannot safely be overlooked.

M.J.M.

272. **How to Use Color in Ice Cream Merchandising and Advertising.** W. A. SUMNER, Univ. of Wisconsin, Madison, Wis. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 53, 1938.

The right colors should be used on delivery trucks, on cartons, in advertising and in the product itself.

In the advertising of food products we should choose colors which suggest "cleanliness." We want to use colors always that are clean. The lighter shades, white, and light shades of yellow and ivory, light green, and light blue, all suggest cleanliness.

In the proper use of color get a good artist who has good taste and then do not insist on cluttering his design of that carton or other design with too much material to compete with it, in terms of letters and lettering.

M.J.M.

273. **Ice Cream Merchandising—Its Possibilities.** F. D. MAYER, Pres., Merchandising Institute of America, Chicago, Ill. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 60, 1938.

The importance of introducing an original idea in selling which will motivate people to buy is essential for satisfactory results. A buying motive

which, psychologically, appeals to many should be created. Various ideas are cited which have been very successful in other industries and the reasons for their success is discussed. M.J.M.

274. The Composition and Serving Temperature as a Means of Increasing Consumer Preference for Ice Cream. W. H. E. Reid, R. J. Drew, and W. S. Arbuckle, Univ. of Missouri, Columbia, Mo. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *4:* 76, 1938.

The authors draw the following conclusions from their experiments:

1. Ice cream containing 14 per cent fat was given the highest consumer preference at ordinary serving temperatures. However, at high serving temperatures, a fat content of 16 per cent seemed most acceptable and at very low serving temperature, 12 per cent fat was preferred.

2. Ice creams containing 13 per cent serum solids had the highest consumer acceptance at serving temperatures of 10° F.

3. With an average serum solids content of 10 per cent, 16 per cent sugar was preferred to other amounts of sugar. However, when the serum solids content was as high as 13.5 per cent, then 14 per cent of sugar was sufficient.

4. Both the composition and serving temperature affect directly the number of dishes of ice cream obtained to the gallon.

5. With increases in either fat or gelatin, the ice creams became more stable at room temperature. The serving temperature is of little significance when the fat or serum solids content is high. However, with low fat and low stabilizer percentages both the serving temperature and the sugar content are of considerable significance.

The results of these investigations indicate definitely that the composition and serving temperature of ice cream are factors that have a decided effect upon consumer acceptance, and that the manufacturers and dispensers may regard them with considerable importance, as a means of increasing consumer preference of their product. M.J.M.

275. Ice Cream Can Analysis. Special Bulletin No. 59. General Survey of the Int. Assn. of Ice Cream Mfrs., 1105 Barr Bldg., Washington, D. C.

The survey of 470 plants making 88,800,000 gallons of bulk ice cream, shows that 46.2 per cent of the ice cream companies reporting use steel cans exclusively while only 8.1 per cent use paper cans entirely. However, small companies use the steel cans and large companies use paper cans. Therefore, a much greater amount of ice cream is packed in paper than the above figures would indicate. Some manufacturers (45.7 per cent) use both paper and steel cans and give the customer the choice. Others use the steel cans as long as they are usable and gradually shift to paper as the steel cans need to be discarded. The 5 gal. size of steel can is used more than the 2½ gal. size but with paper cans the reverse is true.

So far as plant costs are concerned, in the plant only, the cost of the steel cans is less than that of paper. However, the use of paper cans decreases the trucking weight about 30 per cent. In rail shipment of ice cream a very considerable saving in shipping costs can be made by the use of paper cans instead of steel cans. M.J.M.

276. Preliminary Report on Ice Cream Can Survey. O'NEAL M. JOHNSON, Washington, D. C., Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 12, Oct., 1938.

Editors Note: This is a report of the data obtained in the survey reported in Special Bulletin No. 59 and abstracted in the previous abstract.

277. Contamination of Fountain Utensils. ANONYMOUS. Ice Cream Rev., *22:* 8, 94, 1934.

The great majority of ice cream outlets continue to operate with dirty dippers in a manner that leaves the entire ice cream industry open to the threat of investigation by public health authorities and a flood of unfavorable and injurious publicity. J.H.E.

278. Some Causes of High Power Costs. C. T. BAKER, Consulting Engineer, Atlanta, Ga. Ice Cream Rev., *22:* 8, 88, 1939.

In many cases investigated it has been found that the major factor influencing power cost in ice cream factories has been high condensing pressures. Suggestions for reducing condenser pressures are given. J.H.E.

279. Storage of Cream for Ice Cream. J. J. SAMPEY. Abbott's Ice Cream Corp., Philadelphia, Pa. Ice Cream Rev., *22:* 8, 78, 1939.

Since copper contamination accelerates the development of an oxidized flavor in frozen cream during storage it is desirable to determine the amount of copper which cream may have picked up during processing. Several methods proposed for the determination of copper in milk products are discussed. J.H.E.

Editors Note: This article is essentially the same as that of abstract No. 280.

280. Storage of Cream for Ice Cream. J. J. SAMPEY, Abbott's Dairies Inc., Philadelphia, Pa., Ice Cream Trade J., *35:* 3, 40, 1939.

The suitability of various tests which are available for the selection of cream that can be stored satisfactorily without the development of off flavor is discussed.

A test for the determination of copper reported by Ritter in 1936 was described which showed fair agreement with other methods, however it is not satisfactory for high acid cream.

Ten cc. of cream are heated in a test tube to 195° F. by immersion for two to three minutes in a bath of boiling water. (This is to insure freedom from disturbance by bacteria.)

To the heated and cooled sample is added one drop of:

(a) para-amino dimethyl anilin sulphate prepared by dissolving 0.2 gram in 5 cc. water, boiling with bone charcoal and filtering. This is not stable for more than an hour or two.

(b) alcoholic alpha-naphthol, strength not important (5 per cent works well).

(c) 1 per cent hydrogen peroxide.

The exact amount of (a) and (b) is not critical, but the amount of peroxide is.

The sample is then held at incubation temperature and observed for formation of a blue color.

If much copper is present, this will be formed in a few minutes, and if little, three or four hours.

Evaluation of the amount present may be made by performing the test on samples of known freedom from copper as well as on those with definite amounts added. It is stated that the test is sensitive to 1/20 part of copper per million.

Another test reported by Hileman & Webb J. D. S. xx: 47, 1937, seems more applicable to cream.

This depends on the fact that milk or cream contaminated with copper during pasteurization or processing will show an increase in oxidation reduction potential. If the Eh. of the original milk or cream is first determined and the Eh. of the final product be shown to have a rise of Eh. 100 to Eh. 200, such cream should be considered as unsuitable for storage purposes and should be used within a few days of its production.

This method is particularly useful in tracing copper contamination in the flow of milk or cream through plant equipment. W.H.M.

281. Getting that Fresh Fruit Flavor. D. G. SORBER. Food Research Div., U. S. D. A., Ice Cream Rev., *22:* 8, 50, 1939.

The natural fresh fruit flavor and color of fully ripened fruit can be preserved to a very high degree by a new method of preparation and preservation worked out by the U. S. Laboratory of Fruit and Vegetable Chemistry. The method consists of reducing the fruit to a pulp or puree, adding a predetermined amount of sugar or sirup, packaging in tight containers, and freezing rapidly enough to prevent deterioration. A storage temperature of 0° F. is recommended. J.H.E.

282. Merchandising Ice Cream through the Retail Store. HAROLD GOSSER. Bard's Dairy Stores, Pittsburgh, Pa. Ice Cream Rev., *22:* 8, 46, 1939.

This article contains many helpful suggestions for the successful operation of a retail ice cream store. The three most neglected details in store operation are said to be; dirt, stale, skimpy goods, and lack of friendly interest. In these days most employees at the start are not only unskilled and immature but aso have very little idea of discipline, workmanship, or courtesy. Since no one else has done this teaching the operator of stores must follow a well planned personnel program. J.H.E.

283. Ice Cream Cabinet Maintenance. ANONYMOUS. Ice Cream Rev., *22:* 8, 38, 1939.

Directions are outlined for the care and upkeep of electric motors on ice cream cabinets. J.H.E.

284. Save Money by Refining Raw Sugar. ANONYMOUS. Ice Cream Rev., *22:* 7, 56, 1939.

A newly developed refining unit makes it possible for ice cream manufacturers to refine raw sugar into a clear white sugar syrup for use in ice cream. A saving in cost per pound of sugar solids is claimed. J.H.E.

285. Preserving the Freshness of Ice Cream. A. D. BURKE AND R. M. NEWMAN. Ala. Poly. Inst., Auburn, Ala. Ice Cream Rev., *22:* 7, 50, 1939.

Oat flour (Avenex) added to normal ice cream mix at the rate of .5 per cent retarded the development of a stale flavor upon storage. The melting properties of ice cream made from butter as the sole source of fat were improved by the addition of the oat flour. J.H.E.

286. Protection of Ice Cream Against Contamination after Pasteurization. F. E. NELSON, Kans. Agr. Expt. Sta., Manhattan, Kans. Ice Cream Rev., *22:* 7, 46, 1939.

Results indicate that if organisms of the Escherichia-aerobacter group are present in a sample of ice cream, negative to the phosphatase test, they are indicative of contamination following pasteurization. Use of these two determinations in locating sources of contamination in ice cream plants is suggested. J.H.E.

287. Sterile Glassware at the Soda Fountain. ANONYMOUS. Ice Cream Rev., *22:* 7, 42, 1939.

A description is given of a new ultra-violet light sterilizing cabinet for soda fountain glassware. In tests made the bacterial reduction of *E. coli* and *B. subtilis* was 99 per cent. J.H.E.

288. What's the Outlook for Specialized Ice Cream Stores? ANONYMOUS. Ice Cream Rev., *22:* 7, 68, 1939.

Present trend in size and type of retail outlets is away from specialization and toward a return to the old general store concept. J.H.E.

289. How Can Plant Operating Efficiency Be Increased? H. J. BROWN, Goldenrod Ice Cream Co., Chicago, Ill. Ice Cream Rev., *62:* 7, 62, 1939.

Suggestions are given for the control of plant operations leading to greater efficiency and reduced production costs. J.H.E.

290. Practical Savings in Accounting Office Costs—Round Table Discussion. J. S. BLOOM, Abbott's Dairies, Inc., Philadelphia, Pa. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 64, Oct., 1938.

Points stressed in the discussion were the choice of personnel, flow of work, light and air for office workers, office equipment, usefulness of reports and employee relations. M.J.M.

291. Report of Statistical and Accounting Bureau for 1937–38. O'NEAL M. JOHNSON, Washington, D. C. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 61, Oct., 1938.

A tabulation is given of the number of companies and gallonage represented in the expense comparisons of the association. Special surveys made during the year included the retail store analysis, the ice cream can survey, and the labor analysis. The ice cream sales index was also determined. Through August of 1938, a decrease in gallonage of 4.98 per cent was found for the United States, and for Canada, 4.66 per cent. M.J.M.

292. The Influence of New Products on Costs. H. C. THOMAS, Harrington & Company, Dushore Pa. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 55, Oct., 1938.

From 1930 to 1935 the sale of packaged ice cream increased about 50 per cent. The need for getting actual costs on the various products sold is more essential now than ever before. The procedure used by Mr. Thomas was to set up a "Brick and Specialty Department" and charge against the products made in it all necessary costs, as fixed charges, labor, repairs, supplies, etc. The products costs, refrigeration costs, delivery and sales costs, are all charged in direct proportion against this department. In this way the influence of any new product on costs is easily determined. M.J.M.

293. The Use of Product Costs by Management. C. A. ARMITAGE, United Farmers Cooperative Creamery Assn., Charlestown, Mass.

Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 45, Oct., 1938.

The accounting division should prepare "Product Cost" figures accurately and promptly for each month or quarter. The production department has the benefit of comparisons between current costs and those of previous periods, and should work closely with the sales department in an effort to produce at costs which will permit a satisfactory gross profit to be realized. Close cooperation of all departments in the use of "Product Costs" should react to the benefit of the entire business. M.J.M.

294. Practical Savings through the Use of Accounting. P. B. Beck, The Borden Company, New York, N. Y. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 35, Oct., 1938.

The cost of accounting must be met by saving from accounting. Therefore, only those records which are indispensable should be kept.

Detailed expenses as to total and unit cost per gallon for each month must be known. Departmental and divisional costs must also be known. If the average costs are known, then savings can be made when certain cost figures are evidently too high. Suggestions are made for savings in water, power and refrigeration costs. Other preventable costs cited as examples are those for preventable accidents, fires and unnecessary telephone and telegraph expenditures. M.J.M.

295. Eliminating Cost Distortions Due to Seasonal Volume Variations. L. C. Anderson, General Ice Cream Corp., Schenectady, N. Y., Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 25, Oct., 1938.

An accountant should furnish the operating executives, at least monthly, data on the following points:

1. Payroll comparisons, 2. Expense comparisons, 3. Data on volume and price trends, 4. Cost of mix materials, 5, Data on yields and losses, and 6. Data on route operations.

Current data sufficiently reliable to inform the management as to these trends should be sufficient for management to guide operations intelligently. Further data of a non-current nature is also necessary, such as information as to selling prices needed for profitable operations and which items produce the most profits. If different plants are operated, the relative efficiency of the different units must be determined. M.J.M.

296. President's Address. J. L. Bloom, Abbott's Dairies, Inc., Philadelphia, Pa. Proc. 38th Ann. Conv. Int. Assn. of Ice Cream Mfrs., *3:* 7, Oct., 1938.

This address is a discussion of the tendency towards increased governmental regulation of business. The suggestion is made that companies should maintain satisfactory public relations so as to avoid undue difficulties in dealing with employees and consumers. M.J.M.

297. Nutritional and Health Values of Frozen Desserts. W. H. E. Reid, Dairy Dept., Univ. of Mo., Ice Cream Trade J., *35:* 2, 20, 1939.

Attention is directed to the composition of ice cream and the function of the various constituents from a nutritional standpoint. Emphasis is placed on the care exercised in the manufacture of factory made ice cream and on the sanitary quality of the product which results from inspection and supervision of the manufacturing processes. Testimonials from nutrition authorities as to the merits of ice cream as a food are cited.

According to these findings, ice cream possesses the most desirable eating qualities from the standpoint of flavor, body, and texture when served at a temperature of 12 to 14° F. W.H.M.

298. Controlling Oxidized Flavors. A. C. Maach and P. H. Tracy, Dairy Ind., Univ. of Ill., Ice Cream Trade J., *35:* 3, 32, 1939.

The protective action of oat flour (Avenex) in preventing oxidized flavor development in ice cream, sherbets and sweet cream and the effect of the flour on other properties of the ice cream were studied. Results reported by the authors indicate: 1, When added at the rate of 0.3 to 0.5 per cent to vanilla ice cream mix containing 3 p.p.m. copper before freezing, Avenex seemed to protect the ice cream from a stale flavor for two weeks. 2, In strawberry ice cream the protective action of the oat flour was not as great as in vanilla ice cream. Samples which contained 2 and 3 p.p.m. copper were metallic after the first week and more so after two weeks. Samples containing 1 p.p.m. copper were slightly metallic at the end of two weeks. 3, In strawberry sherbet oat flour was reported to be helpful in flavor and body improvement. 4, Oat flour added to sweet cream at the rate of 1.5 per cent in the form of a gruel before pasteurization afforded rather good protection to the cream stored in the frozen state. Dry oat flour added to this cold cream was of no value. 5, Oat flour when added at the rate of .5 per cent to the ice cream mix had no effect on titratable acidity or pH of the mix, doubled the viscosity over that of the control, did not effect the melt down properties of the ice cream and did not appreciably affect the time required to get 100 per cent overrun. Difficulty was reported in making sediment tests on mixes containing oat flour. W.H.M.

MILK

299. The Influence of Heavy Metals on the Enzymic Reactions of Milk. W. Ritter, Fifth International Congress for Technique and Chemistry of Agricultural Industries, Scheveningen, *2:* 297–306, 1937.

The influence of copper and iron on the aldehyde-reductase (xanthine-oxidase) of milk and cream, as well as the occurrence of positive peroxidase reactions in heated milk and cream and their influence on the oxidation constancy of milk are treated in this paper. Small amounts of copper are sufficient to prolong very much the methylene blue reduction time of a mixture of milk and formaldehyde. All copper containing alloys (brass, bronze, German silver, monel metal) produce the same effect, though in a somewhat lesser degree than pure copper.

In just the same way the presence of small amounts of copper is able to develop a positive peroxidase reaction in milk. The effect of copper on this reaction works about parallel with its effect on the flavor of the milk, which becomes tallowy. Nickel and iron also produce a bad flavor in the milk, but they do not alter its enzymic reactions. W.R.

300. The Influence of Pasteurization-Temperature on Certain Properties of Milk. W. RITTER. Schweiz. Milchzeitung 1937 (special number published in honor of the 70th birthday of Prof. Dr. Rob. Burri).

Besides the well-known effects of increased temperature on milk (destruction of bacteria and of enzymes, coagulation of the albumin, alteration of the composition of milk-salts, of cream formation, of color, smell and flavor and so on), heating also influences to a great extent the reaction of milk in oxidation and reduction processes. The following points are to be taken into account: According to investigations of Burri and Kursteiner of 25 years ago, milk submitted to high heating is able to reduce methylene blue under exclusion of oxygen. This effect is perfectly independent of bacterial and enzymic activity. Differences occur in determining the reduction time of bacteria cultures inoculated on milk heated at lower temperature, for instance such as has been submitted to holding pasteurization or to a temperature of 80 or 90° C. Methylene blue is more rapidly reduced in milk heated at high temperature than in milk submitted to holding pasteurization. On the other hand, the influence of copper on the reappearance of the color on shaking the samples is more perceptible in milk submitted to holding pasteurization than in milk heated at 80 or 90° C. Peroxydase- and nadi-reaction on milk heated at high temperature, if founded on purely chemical reasons (presence of small amounts of copper), is all the more feeble as the heating has been more intense or longer. This dependence becomes also evident when a small amount of raw milk is added to heated milk. The effect of copper and iron on the developing of tallowy flavor in milk heated at different degrees is analogous.

Milk that is liable to become tallowy shows the preventive or at least checking effect of intense heating on this defect. These questions are of great importance for condensed milk and milkpowder, which also reveal

different constancy against oxidation, according to the degree of heating; this circumstance may lead to the abolition of the still frequent use of copper vacuum apparatus. When butter is prepared with acid cream, the important fact appears that the heating of the cream at 90° C. in flash-pasteurizers prevents the developing of fishy flavor during the storing, while heating at 80–84° C. is not sufficient for that effect. Butterfat obtained at low temperature is much less constant against oxidation than properly cooked butter. W.R.

301. Off-Flavors in Winter Milk. H. J. BRUECKNER, Am. Pro. Rev. 85: *14*, 378–79, 1938.

The period of year during which cows are stabled is the period during which disagreeable flavors occur most frequently in milk. Feeds imparting off flavors should be fed only after milking. Most grain mixtures may be fed during milking. Air impregnated with feed odors and breathed by the cow may cause transference of the odor to milk before it is drawn. Ventilation will remove much of this difficulty.

A common abnormal flavor occurring especially in raw milk and cream is that of bitterness or rancidity. This develops apparently more frequently with cows late in the lactation period and especially with those in poor condition through underfeeding and with those who are low producers. In extreme cases the flavor develops in four to six hours, but usually 12 or more hours are required. The defect is due to the action of the enzyme, lipase. Tests for amounts of chlorides present will give a clue to whether the milk will become rancid but if mastitis is present the test interpretation is misleading. Flash heating of milk to 120°–130° F., cooling to 75–85° F. before separation to partially restore viscosity will aid in prevention of the flavor in cream. Pasteurization before the flavor develops will prevent the flavor in milk. This process will not eliminate the flavor once it is present.

Oxidized or tallowy flavor is chiefly caused by passing warm or hot milk at the farm or factory over copper, iron, copper alloys as bronze, brass, ambrac, or German or nickel silver. The only remedy seems to be the elimination of any equipment containing copper in the surface exposed to milk. Other factors, less easily controlled, are the greater susceptibility of some milk to develope the defect, exposure of milk to sunlight, chlorine sterilization of utensils, and the amount of air in milk. P.S.L.

302. Revision of Methods and Standards for Certified Milk. J. HOWARD BROWN, Johns Hopkins Univ., Baltimore, Md. Chairman, Committee on Methods and Standards of the American Association of Medical Milk Commissions, Am. J. Pub. Health, *29:* 4, 355, 1939.

Topics discussed are the bacterial plate count using an improved medium, coliform counts and their significance, the spore test, identification of hemo-

lytic streptococci and other tests. The author urges health officials to take a broader interest in milk than to make it safe and points out that there are possibilities of improving the flavor and nutritive quality of milk.

M.W.Y.

303. Place of the Methylene Blue and Resazurin Reduction Tests in a Milk Control Program. C. K. JOHNS, Dominion Dept. of Agr., Ottawa, Ontario, Canada. Am. J. Public Health *29:* 3, 239, 1939.

In routine testing of incoming supplies, the author believes that the methylene blue reduction test has many advantages over the usual counting methods. Inversion of tubes every two hours yielded results in good agreement with those from more frequent mixing and greatly improved the accuracy of the test as compared to the standard method. The resazurin one-hour test did not indicate the bacterial content as reliably as did the modified methylene blue reduction test. M.W.Y.

304. Sanitary Dispensing of Beverages. EDITORIAL, Am. J. Pub. Health, *29:* 2, 166, 1939.

Devices have been perfected for the hygienic dispensing of pasteurized milk from milk containers in restaurants. After more than three years' study, seven such devices have been approved by the Health Department of New York City. The essential features are simplicity and ease of operation. They should be made of materials that not only resist contamination and are non-toxic, but are so constructed that they may be frequently and easily disassembled and quickly and effectively cleansed or sterilized in boiling water or appropriate chemical solutions. M.W.Y.

305. Value of Phosphatase as an Indicator of Pasteurization. F. W. GILCREAS, Div. of Laboratories and Res., N. Y. State Dept. of Health, Albany, N. Y. Am. J. Pub. Health, *29:* 2, 158, 1939.

The phosphatase test gives precise information concerning the pasteurization of milk. Prolonged preheating or slight over-heating may reduce the phosphatase content of the milk sufficiently to make it impossible for the laboratory test to detect technical shortening of the holding time by 5 minutes or the addition of 0.1 per cent of raw milk.

Cream separated from raw milk and then pasteurized gives a reaction of complete treatment. Cream separated from pasteurized milk either by gravity or by mechanical means tends to have a high phenol value indicative of under-pasteurization. The tendency for the phosphatase value of cream to increase beyond that of the original milk is discussed, and the statement made that it may become necessary to establish a separate code of readings for cream. M.W.Y.

MISCELLANEOUS

306. The Influences of Cleaning Materials on some Metals. W. RITTER AND THS. NUSSBAUMER: Schweiz. Milchzeitung No. 51, 53, 55, 56, 57, 1937.

It was found that no perceptible corrosion of the metals employed in dairy industry is produced by the commonly used cleaning materials "Neomoscan," "P_3-zinnfest" (which does not attack tin) and "P_3-sterile." On the other hand the material called "Try" which coontains trisodium-phosphate effects a strong corrosion on aluminium and its alloys on account of its too low sodium silicate content. The milk-stone solvent "Tartrex" which consists mainly of winestone (acid potassium-tartrate) distinctly attacked tinned metals, while it did not produce much effect on aluminium and its alloys. On examining metal combinations in conducting connection, we obtained weak currents when dissolvents containing sodium silicate were used and rather strong currents with soda alone. The latter currents produced accordingly strong corrosions. Relatively strong currents which scarcely slackened after 24 hours resulted when metals in conducting connection were immersed in Tartrex solutions. A stronger corrosion of the baser metals was quite apparent, while the more precious metals were less attacked in corresponding experiments with single metals. Conditions were particularly marked in a combination of copper and aluminium. While tinned material was distinctly attacked by Tartrex alone, the corrosion was still stronger in tinned material in conducting connection with stainless steel. Tinned material proved baser at first in Tartrex solutions than aluminium, but the direction of the current changed after a few hours. None of the tested cleaning materials produced a perceptible corrosion on steel alone or combined with other metals. Anode oxidized aluminium and its alloys were attacked if the sodium silicate content of the solutions was too low. W.R.

307. Some Properties of Lactose. W. RITTER. Schweiz, Milchzeitung No. 71–74, 1937.

Properties, formation and transformations of the three well-known forms of lactose (α-lactose-hydrate, α-lactose-anhydride and β-lactose-anhydride) are discussed and their importance for dairy problems (sandiness in sweetened condensed milk and ice cream, action in milk powder) is made known. It follows, from the conditions of formation, that the lactose in the cooking residue of butter is certainly present in the form of β-lactose-anhydride. W.R.

PHYSIOLOGY

308. Roentgen Studies of the Mechanism Involved in Sperm Transportation in the Female Rabbit. ROBERT H. KREHBIEL AND HERMAN

P. CARSTENS, Dept. of Anatomy and Physiology, Univ. of Ill. Am. J. Physiol. *125*, 3, 571, 1939.

Transfer of methylene blue, janus green B and iodochlorol through the reproductive system of oestrus rabbits have been observed after artificial stimulation of the vulva. These dyes reach the tubal end of the uterine horns 2 to 5 minutes after the start of stimulation. The observed transfer of fluids into and through the uterus may be interpreted as being similar to the transfer of spermatozoa and would indicate that spermatozoa are passive in their movement to the utero-tubal junction in animals which have an extended heat period. Despite the continued activity on the part of the vagina and uterus over a period of an hour the authors were unable to detect any fluid in the tubes. In fact, pressures were created at the junctions of the uterus and tubes which would be sufficient to force the liquid into the tubes if a sphincter were not present. D.L.E.

309. **Gonadotropic Extracts from Green Leaves.** MAURICE H. FRIEDMAN AND GERTRUDE S. FRIEDMAN, Dept. of Physiology, Univ. of Pennsylvania Medical School. Amer. J. Physiol. *125*, 3, 486, 1939.

Because of the protein nature of the pituitary hormones and the fact that qualitative or quantitative restriction of dietary protein is known to interfere with reproduction, the authors decided to test the hypothesis that dietary proteins contain indispensable precursors for the formation of the pituitary proteins. They found that it is possible to extract from the dried leaves of immature grasses a fraction which is capable of producing ovulation upon injection into rabbits. The observed chemical properties of this fraction simulate the properties of the gonadotropic material in anterior pituitary extracts. D.L.E.